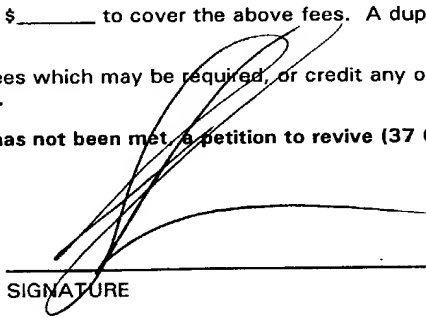


FORM-PTO-1390 (Rev. 12-29-99)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	
<b>TRANSMITTAL LETTER TO THE UNITED STATES          DESIGNATED/ELECTED OFFICE (DO/EO/US)          CONCERNING A FILING UNDER 35 U.S.C. 371</b>		ATTORNEY'S DOCKET NUMBER 012627-025 U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) <b>UNASSIGNED 09/936738</b>	
INTERNATIONAL APPLICATION NO. PCT/EP00/02330	INTERNATIONAL FILING DATE 21 SEPTEMBER 2000	PRIORITY DATE CLAIMED 19 MARCH 1999 31 DECEMBER 1999	
TITLE OF INVENTION Method for Identifying Organisms by Means of Comparative Genetic Analysis and Primers and Hybridization Probes for Carrying out this Method			
APPLICANT(S) FOR DO/EO/US Hans SCHACKERT et al.			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.			
2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.			
3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).			
4. <input type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.			
5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))			
a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).			
b. <input type="checkbox"/> has been transmitted by the International Bureau.			
c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)			
6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).			
7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))			
a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).			
b. <input type="checkbox"/> have been transmitted by the International Bureau.			
c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.			
d. <input checked="" type="checkbox"/> have not been made and will not be made.			
8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).			
9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).			
10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).			
<b>Items 11. to 16. below concern other document(s) or information included:</b>			
11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.			
12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.			
13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.			
<input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.			
14. <input type="checkbox"/> A substitute specification.			
15. <input type="checkbox"/> A change of power of attorney and/or address letter.			
16. <input checked="" type="checkbox"/> Other items or information:			
International Search Report International Preliminary Examination Report PCT Request form (PCT/RO/101) (8) sheets of Drawings (48) Sheets (List of species sequences)			

U.S. APPLICATION NO. (If known, see 37 CFR 1.50) <b>UNASSIGNED 09/936738</b>		INTERNATIONAL APPLICATION NO. <b>PCT/EP00/02330</b>		ATTORNEY'S DOCKET NUMBER <b>012627-025</b>	
17. <input type="checkbox"/> The following fees are submitted:				<b>CALCULATIONS</b>	<b>PTO USE ONLY</b>
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b>  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$1,000.00 (960)  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$860.00 (970)  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$710.00 (958)  International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$690.00 (956)  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00 (962)  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>\$ 860.00</b>	
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>				\$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	52 -20 =	32	X\$18.00 (966)	\$ 576.00	
Independent Claims	21 -3 =	18	X\$80.00 (964)	\$ 1440.00	
Multiple dependent claim(s) (if applicable)			+ \$270.00 (968)	\$	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$ 2016.00</b>	
Reduction for 1/2 for filing by small entity, if applicable (see below).				\$	
<b>SUBTOTAL =</b>				<b>\$ 2876.00</b>	
Processing fee of \$130.00 (156) for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>				\$	
<b>TOTAL NATIONAL FEE =</b>				<b>\$ 2876.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +				\$	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$ 2876.00</b>	
				<b>Amount to be: refunded</b>	\$
				<b>charged</b>	\$
<p>a. <input type="checkbox"/> Small entity status is hereby claimed.</p> <p>b. <input checked="" type="checkbox"/> A check in the amount of \$ <u>2876.00</u> to cover the above fees is enclosed.</p> <p>c. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$_____ to cover the above fees. A duplicate copy of this sheet is enclosed.</p> <p>d. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u>. A duplicate copy of this sheet is enclosed.</p> <p><b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b></p> <p>SEND ALL CORRESPONDENCE TO:</p> <p style="margin-left: 40px;">Teresa Stanek Rae BURNS, DOANE, SWECKER &amp; MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620</p> <div style="text-align: right; margin-top: 20px;"> _____ SIGNATURE  _____ NAME  _____ 30 427 REGISTRATION NUMBER</div>					

Patent

Attorney's Docket No. 012627-025

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of	)	
	)	
Prof. Dr. Hans SCHACKERT et al.	)	Group Art Unit: Unassigned
	)	
Application No.: Unassigned	)	Examiner: Unassigned
(Corresponding to PCT/EP00/02330	)	
	)	
International Filing Date: 16 March 2000	)	
	)	
For: METHOD FOR IDENTIFYING	)	
ORGANISMS BY MEANS OF	)	
COMPARATIVE GENETIC	)	
ANALYSIS AND PRIMERS AND	)	
HYBRIDISATION PROBES FOR	)	
CARRYING OUT THIS METHOD	)	
	)	

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination, kindly amend the above-identified application as follows:

**IN THE CLAIMS:**

Kindly replace claims 1-45 and 47-52 as follows:

1. (Amended) A method of identifying organisms by comparative genetic analysis, wherein the coding and/or non-coding areas and/or functionally significant areas of highly conserved genes and/or their homologous genes and/or their cDNA copies and/or their pseudogenes are amplified using PCR and are subsequently genotyped and analyzed.

2. (Amended) The method according to claim 1, wherein one primer pair each is used for each specific segment of the highly conserved gene, which is located in the highly conserved exon region and/or non-coding areas and/or functionally significant areas and/or in the 5'- or 3'-untranslated area of the gene and binds in as many studied species DNAs as possible, preferable in all studied species DNAs, and enables the amplification of the corresponding gene area.

3. (Amended) The method according to claim 1, wherein the coding and/or non-coding areas located between the primers and being either highly variant intron regions and/or variant exon regions or 5'- or 3'-untranslated areas of the gene, are analyzed as regards their sequence and identified by comparison with the species-specific sequence variants.

4. (Amended) The method according to claim 1, wherein either the sense strand or the antisense strand of any species DNA or also their PCR copies are used for the identification.

5. (Amended) The method according to claim 1, wherein animals are identified.



6. (Amended) The method according to claim 1, wherein vertebrates are identified.
7. (Amended) The method according to claim 1, wherein mammals are identified.
8. (Amended) The method according to claim 1, wherein plants are identified.
9. (Amended) The method according to claim 1, wherein genotyping is carried out by DNA sequencing, any hybridization method, restriction fragment length analyses, chromatographic methods, spectroscopic and mass-spectroscopic methods, allele-specific PCR or by other methods suitable for detecting DNA sequence variants.
10. (Amended) The method according to claim 1, wherein exon and/or intron areas as well as functionally significant areas of the highly conserved tumor suppressor gene PTEN/MMAC1 and its homologues are used for amplification and subsequent genetic analysis.
11. (Amended) The method according to claim 1, wherein cDNA copies of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.

12. (Amended) The method according to claim 1, wherein pseudogenes or segments of pseudogenes of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.

13. (Amended) The method according to claim 1, wherein exons arranged next to the PTEN/MMAC1 gene and its homologues and/or the parts of the introns following the exons are analyzed genetically.

14. (Amended) The method according to claim 1, wherein the exon regions 1 and 2 and/or 3 and 4 and/or 4 and 5 and/or 5 and 6 and/or 6 and 7 and/or 7 and 8 and/or 8 and 9 with the enclosed intron regions 1 and/or 2 and/or 3 and/or 4 and/or 5 and/or 6 and/or 7 and/or 8 as well as the 5'- and 3'-untranslated regions of the PTEN/MMAC1 gene and their homologues are used for the genetic analysis.

15. (Amended) The method according to claim 1, comprising selecting areas of highly conserved genes and/or pseudogenes and their homologues, constructing suitable oligonucleotides as primers which bind to the corresponding complementary coding and/or non-coding areas and/or functionally significant areas, amplifying them by means of a suitable technique and comparatively analyzing the sequence of the corresponding coding and/or non-coding area of various species by genetic analysis.

16. (Amended) The method according to claim 15, wherein areas of the PTEN/MMAC1 gene and/or the pseudogene and their homologues are selected.

17. (Amended) The method according to claim 15, wherein differing sequence segments of each individual exon, intron or untranslated region of the PTEN/MMAC1 gene and their homologues or the corresponding cDNA are selected.

18. (Amended) The method according to claim 1, wherein genotyping of pig DNA which is obtained from foodstuffs, is carried out on the basis of the gene sequence variant of PTEN/MMAC1 containing a 9-base pair long deletion.

19. (Amended) An oligonucleotide primer for the PCR and the sequencing of exon 1 and/or 5'-untranslated region of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

PTENex1-401 sense

5'-cccttctactgcctcca -3'

PTENex1 -465 sense

5'- gggagggggtctgagt -3'

PTENex1 ATG sense

5'- atgacagccatcatcaaaga -3'

PTENex1 R antisense

5'- aggtcaagtctaagtcgaatc -3'

20. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 2 of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

PTENex2F sense

5'- atatttatccaaacattattgctat -3'

PTENex2R antisense

5'- ct tactacatcatcaatattgttcc -3'

21. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 4, intron 4 and exon 5 of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

Zoo43sUV sense

5'- tgtgctgagagacattatgac -3'

SPL5 sense

5'- aaatttaattgcagaggt -3'

Zoo44aRV antisense

5'- ttgtctctggctccttacttc -3'

22. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 5 of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

PTEN se sense

5'- atcttgaccaatggctaagtg -3'

Zoo44aRV antisense

5'- ttgtctctggctccttacttc -3'

23. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 6 of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

PTENex6F sense

5'- gga gta act att ccc agt cag ag -3'

PTENex6R antisense

5'- gca agt tcc gcc act gaa -3'

24. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 7 of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

PTENex7F sense

5'- cct cag ttt gtg gtc tgc ca -3'

PTENex7R antisense

5'- c ctt ttt tag cat ctt gtt ctg ttt -3'

25. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 8 of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

PTENex8F sense

5'- caa aat gtt tca ctt ttg ggt aaa -3'

PTENex8R antisense

5'- taa aat ttg gag aaa agt atc ggt t -3'

26. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 9 of the PTEN/MMAC1 gene and its homologues, comprising the following sequences:

5'- gtg aag ctg tac ttc aca aaa ac -3'

5'- aaa aaa att cag act ttt gta att tg -3'

28. (Amended) The method according to claim 1, wherein the oligonucleotides

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat t -3',

5' - cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc t -3'

are used for the amplification.

29. (Amended) The method according to claim 1, wherein the oligonucleotides  
*sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gaa -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gag -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gat -3',

*antisense:*

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt a -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt g -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt t -3'

are used for the amplification.

30. (Amended) The method according to claim 1, wherein DNA sequencing  
methods are used for the genetic analysis.



31. (Amended) The method according to claim 1, wherein DNA sequencing techniques are used in the genetic analysis for the PTEN/MMAC1 and/or its pseudogenes and their homologues.

32. (Amended) The method of distinguishing the DNA of various species, wherein at least one hybridization probe pair is used, the melting points of different combinations are determined and compiled for each species into a panel.

33. (Amended) The method of distinguishing the DNA of various species, wherein at least one hybridization probe pair is used and at least one gene segment is amplified, differing hybridization probe pairs hybridize to different gene segments, and the melting points of the different combinations are determined and compiled for each species into a panel and/or compared with this panel for the purpose of identification.

34. (Amended) The method of distinguishing the DNA of different species according to claim 33, wherein at least one hybridization probe pair is used and at least one gene segment of at least one species is amplified, differing hybridization probe pairs hybridize to different gene segments of various species, and the melting points of the different combinations are determined and compiled for each species into a panel and/or compared with this panel for the purpose of identification.

35. (Amended) The method of distinguishing the DNA of various species according to claim 33, wherein at least two hybridization probes of SEQ Nos. 3 to 8 are used, the melting points of different combinations are determined and compiled for each species into a panel.

36. (Amended) The method according to claim 33, wherein the species differentiation of pig DNA from various other species is made using the hybridization probe pair A1/A2 as the hybridization probe pair.

37. (Amended) The method according to claim 33, wherein the hybridization probes are used in combinations C1/C2; A1/B2; A1/A2; C1/A2; B1/B2; B1/A2 for the species differentiation between various species.

38. (Amended) LightCycler hybridization probes for exon 5, comprising the sequences:

A1: 5'- tgc ata ttt gtt tca tcc ggg caa att -fluorescein -3'

A2: 5'- LC Red 705 - tta aag gca caa gat ttc tat ggg ga - ph -3'

B1: 5'- tgc ata ttt att aca tcg ggg caa att -fluorescein -3'

B2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

C1: 5'-tgc ata ttt gtt aca tcg ggg taa att fluorescein -3'

C2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

39. (Amended) LightCycler hybridization probes for exon 6, comprising the sequences

PTENex6FL

5'- tca tct gga tta tag acc agt ggc act - fluorescein -3'

PTENex6LC 640

5'- LC Red 640 - ttc aca aga tga tgt ttg aaa cta ttc caa- ph -3'

PTENex6F\*

5'- gtg cca ctg gtc tat aat cca gat- fluorescein -3'

PTENex6L\* 705

5'- LC Red 705- ttc ttt aac agg tag cta taa taa tac aca ta- ph -3'

40. (Amended) LightCycler hybridization probes for exon 7, comprising the sequences

PTENex7F\*

5'- taa agg tga aga tat att cct cca att ca - fluorescein -3'

PTENex7L\*640

5'-LC Red 640- acc cac acg acg gga aga caa g - ph -3'

PTENex7 FL

5'-ggtaacggctgagggaactcaaagtac - fluorescein -3'

PTENex7 LC (705-labeled)

5'-LC Red 705- tgaactgtcttcccgtcgtgtgg- ph -3'

41. (Amended) LightCycler hybridization probes for exon 8, comprising the following sequences

PTENex8F\*

5'- tga caa gga ata tct agt act tac ttt aac aaa-fluorescein -3'

PPTENex8L\* 705

5'-LC Red 705 - ctt gac aaa gca aat aaa gac aaa gc- ph -3'

PTENex8 FLU

5' - tgctatcgatttcttgatcacatagacttccatttt - fluorescein -3'

PTENex8 LCR (640-labeled)

5'-LC Red 640- actttttctgagggttcctctggtcctggat - ph -3'

42. (Amended) [The] LightCycler hybridization probes for exon 9, comprising the following sequences

PTENex9 FL

5'-aac atc tgg tgt tac aga agt tga act gct- fluorescein -3'

PTENcx9 LC 640

5'-LC-640- cct ctg gat ttg acg gct cct cta ct - ph -3'

43. (Amended) Hybridization probe pair AI/A2: specific to PTEN pseudogene pig, comprising

SEQ No. 3 A1: 5'- tgc ata ttt gtt tca tcc ggg caa att -fluorescein -3'

SEQ No. 4 A2: 5'-LC Red 705- tta aag gca caa gat ttc tat ggg ga - ph -3'

44. (Amended) Hybridization probe pair B1/B2: specific to pseudogene man, comprising

SEQ No. 5 B1: 5'- tgc ata ttt att aca tcg ggg caa att -fluorescein -3'

SEQ No. 6 B2: 5'-LC Red 640- aag gca caa gag gcc cta gat ttc ta -ph -3'

45. (Amended) Hybridization probe pair C1/C2: specific to PTEN pseudogene man (C2) and homologue of pig (C1) comprising

SEQ No. 7 - C1: 5'- tgc ata ttt gtt aca tcg ggg taa att - fluorescein -3'

SEQ No. 8 - C2: corresponds to probe B2.

47. (Amended) DNA sequences of homologues of the PTEN/MMAC1 gene and/or of homologues of the PTEN/MMAC1 pseudogene, which are compiled in the annex under "list of species sequences", which as compared to the PTEN/MMAC1 gene and/or the PTEN/MMAC1 pseudogene comprise genetic variants comprising base substitutions and/or insertions and/or deletions and are suited for identifying corresponding species.

48. (Amended) A kit for carrying out the method according to claim 1, comprising:

- a) one or more vessels comprising PCR and/or sequencing oligonucleotides binding to highly conserved genes, the oligonucleotides being optionally labeled radioactively or by means of a dye or in another way,
  - b) vessels having further common reagents for DNA amplification and/or DNA analysis,
- and
- c) a vessel containing a control DNA which is suited for testing the oligonucleotides and the reaction conditions.

49. (Amended) The kit according to claim 48, comprising:

- a) one or more vessels with PCR and/or sequencing oligonucleotides.

50. (Amended) The kit for identifying species for carrying out the method according to claim 1, comprising:

- a) a vessel having an oligonucleotide pair comprising the following sequences:  
5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3' and 5'- cag  
gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',
- b) two vessels with one of the following sequencing oligonucleotides each,  
these oligonucleotides being optionally labeled radioactively or by means of  
a dye or in another way:  
5'- cag gaa aca gct atg ac -3' and  
5'- cga cgt tgt aaa acg acg gcc agt -3',
- c) a vessel containing a control *DNA*, which is suited for testing the  
oligonucleotides and the reaction conditions.

51. (Amended) The kit (Light Cycler Kit) for carrying out the method according to claim 32, comprising

- a) one or more vessels containing PCR primers and hybridization probes,  
which bind to highly conserved genes, the hybridization probes being  
optionally labeled by means of a dye,
  - b) vessels containing further common reagents for DNA amplification and/or  
DNA analysis,
- and



- c) a vessel containing a control DNA which is suited for testing the oligonucleotides and the reaction conditions.

52. (Amended) The kit (Light Cycler Kit) for carrying out the method according to claim 32, comprising:

- a) one or more vessels with PCR primers and hybridization probes.

Application No.  
Attorney's Docket No. 012627-025  
Page 20

**REMARKS**

Entry of the foregoing amendments is respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: \_\_\_\_\_

Teresa Stanek Rea  
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(703) 836-6620

**Date: September 17, 2001**

**Attachment to Preliminary Amendment dated XXX****Marked-up Claims 1-45 and 47-52**

1. (Amended) A method of identifying organisms by comparative genetic analysis, [characterized in that] wherein the coding and/or non-coding areas and/or functionally significant areas of highly conserved genes and/or their homologous genes and/or their cDNA copies and/or their pseudogenes are amplified using PCR and are subsequently genotyped and analyzed.
  
2. (Amended) The method according to claim 1, [characterized in that] wherein one primer pair each is used for each specific segment of the highly conserved gene, which is located in the highly conserved exon region and/or non-coding areas and/or functionally significant areas and/or in the 5'- or 3'-untranslated area of the gene and binds in as many studied species DNAs as possible, preferable in all studied species DNAs, and enables the amplification of the corresponding gene area.
  
3. (Amended) The method according to [claims 1 and 2] claim 1, [characterized in that] wherein the coding and/or non-coding areas located between the primers and being either highly variant intron regions and/or variant exon regions or 5'- or 3'-untranslated areas of the gene, are analyzed as regards their sequence and identified by comparison with the species-specific sequence variants.

**Attachment to Preliminary Amendment dated XXX**

**Marked-up Claims 1-45 and 47-52**

4. (Amended) The method according to [claims 1 to 3] claim 1, [characterized in that] wherein either the sense strand or the antisense strand of any species DNA or also their PCR copies are used for the identification.

5. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that preferably] wherein animals are identified.

6. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that preferably] wherein vertebrates are identified.

7. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that preferably] wherein mammals are identified.

8. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that preferably] wherein plants are identified.

9. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that] wherein genotyping is carried out by DNA sequencing, any hybridization [methods] method, restriction fragment length analyses, chromatographic methods, spectroscopic and

**Attachment to Preliminary Amendment dated XXX**

**Marked-up Claims 1-45 and 47-52**

[in particular] mass-spectroscopic methods, allele-specific PCR or by other methods suitable for detecting DNA sequence variants.

10. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that] wherein exon and/or intron areas as well as functionally significant areas of the highly conserved tumor suppressor gene PTEN/MMAC1 and its homologues are used for amplification and subsequent genetic analysis.

11. (Amended) The method according to [claims 1 to 4] claim 1, wherein cDNA copies of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.

12. (Amended) The method according to [claims 1 to 4] claim 1, wherein pseudogenes or segments of pseudogenes of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.

13. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that preferably] wherein exons arranged [side by side of] next to the PTEN/MMAC1 gene and its homologues and/or the parts of the introns following the exons are analyzed genetically.

**Attachment to Preliminary Amendment dated XXX**

**Marked-up Claims 1-45 and 47-52**

14. (Amended) The method according to [claims 1 to 4] claim 1, [characterized in that] wherein the exon regions 1 and 2 and/or 3 and 4 and/or 4 and 5 and/or 5 and 6 and/or 6 and 7 and/or 7 and 8 and/or 8 and 9 with the enclosed intron regions 1 and/or 2 and/or 3 and/or 4 and/or 5 and/or 6 and/or 7 and/or 8 as well as the 5'- and 3'-untranslated regions of the PTEN/MMAC1 gene and their homologues are used for the genetic analysis.

15. (Amended) The method according to [claims 1 to 4] claim 1, [characterized by] comprising selecting areas of highly conserved genes and/or pseudogenes and their homologues, constructing suitable oligonucleotides as primers which bind to the corresponding complementary coding and/or non-coding areas and/or functionally significant areas, amplifying them by means of a suitable technique and comparatively analyzing the sequence of the corresponding coding and/or non-coding area of various species by genetic analysis.

16. (Amended) The method according to claim 15, [characterized in that] wherein areas of the PTEN/MMAC1 gene and/or the pseudogene and their homologues are selected.

17. (Amended) The method according to [claims 15 and 16] claim 15, [characterized in that] wherein differing sequence segments of each individual exon, intron

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or untranslated region of the PTEN/MMAC1 gene and their homologues or the corresponding cDNA are selected.

18. (Amended) The method according to [claims 1 to 17] claim 1, [characterized in that] wherein genotyping of pig DNA which is obtained [preferably] from foodstuffs, is carried out on the basis of the gene sequence variant of PTEN/MMAC1 containing a 9-base pair long deletion.

19. (Amended) An oligonucleotide primer for the PCR and the sequencing of exon 1 and/or 5'-untranslated region of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

PTENex1-401 sense

5'-cccttctactgcctcca -3'

PTENex1 -465 sense

5'- gggagggggtctgagt -3'

PTENex1 ATG sense

5'- atgacagccatcatcaaaga -3'

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PTENex1 R antisense

5'- aggtcaagtctaagtcgaatc -3'

20. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 2 of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

PTENex2F sense

5'- atatttatccaaacattattgctat -3'

PTENex2R antisense

5'- cttactacatcatcaatattgttcc -3'

21. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 4, intron 4 and exon 5 of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

Zoo43sUV sense

5'- tgtgctgagagacattatgac -3'

SPL5 sense

5'- aaatttaattgcagaggt -3'



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Zoo44aRV antisense

5'- ttgtctctggctccttacttc -3'

22. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 5 of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

PTEN se sense

5'- atcttgaccaatggctaagtg -3'

Zoo44aRV antisense

5'- ttgtctctggctccttacttc -3'

23. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 6 of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

PTENex6F sense

5'- gga gta act att ccc agt cag ag -3'

PTENex6R antisense

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5'- gca agt tcc gcc act gaa -3'

24. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 7 of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

PTENex7F sense

5'- cct cag ttt gtg gtc tgc ca -3'

PTENex7R antisense

5'- c ctt ttt tag cat ctt gtt ctg ttt -3'

25. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 8 of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

PTENex8F sense

5'- caa aat gtt tca ctt ttg ggt aaa -3'

PTENex8R antisense

5'- taa aat ttg gag aaa agt atc ggt t -3'

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26. (Amended) The oligonucleotide primer for PCR and the sequencing of exon 9 of the PTEN/MMAC1 gene and its homologues, [characterized by] comprising the following sequences:

PTENex9F sense

5'- gtg aag ctg tac ttc aca aaa ac -3'

PTENex9tga antisense

5'- aaa aaa att cag act ttt gta att tg -3'

27. (Amended) The method according to [claims 1 to 17] claim 1, [characterized in that for] wherein the DNA amplification involves a mixture of oligonucleotides [is used] which differ at the 3' region of the oligonucleotide as regards its length by one or more nucleotides or which differ as regards their nucleotide sequence at the 3' end of the oligonucleotide at one or more positions.

28. (Amended) The method according to [claims 1 to 17 and 26] claim 1, wherein the oligonucleotides

*sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',

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5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat t -3',

*antisense:*

5' - cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc t -3'

are used for the amplification.

29. (Amended) The method according to [claims 1 to 17 and 26] claim 1,  
wherein the oligonucleotides

*sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gaa -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gag -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gat -3',

*antisense:*

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt a -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt g -3',

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5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt t -3'

are used for the amplification.

30. (Amended) The method according to [claims 1 to 17] claim 1,  
[characterized in that] wherein DNA sequencing methods are used for the genetic analysis.

31. (Amended) The method according to [claims 1 to 17] claim 1,  
[characterized in that] wherein DNA sequencing techniques are used in the genetic analysis  
for the PTEN/MMAC1 and/or its pseudogenes and their homologues.

32. (Amended) The method of distinguishing the DNA of various species,  
[characterized in that] wherein at least one hybridization probe pair is used, the melting  
points of different combinations are determined and compiled for each species into a panel.

33. (Amended) The method of distinguishing the DNA of various species,  
[characterized in that] wherein at least one hybridization probe pair is used and at least one  
gene segment is amplified, differing hybridization probe pairs hybridize to different gene  
segments, and the melting points of the different combinations are determined and compiled

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for each species into a panel and/or compared with this panel for the purpose of identification.

34. (Amended) The method of distinguishing the DNA of different species according to claim 33, [characterized in that] wherein at least one hybridization probe pair is used and at least one gene segment of at least one species is amplified, differing hybridization probe pairs hybridize to different gene segments of various species, and the melting points of the different combinations are determined and compiled for each species into a panel and/or compared with this panel for the purpose of identification.

35. (Amended) The method of distinguishing the DNA of various species according to [claims 33 and 34] claim 33, [characterized in that] wherein at least two hybridization probes of SEQ Nos. 3 to 8 are used, the melting points of different combinations are determined and compiled for each species into a panel.

36. (Amended) The method according to [claims 33 and 34] claim 33, [characterized in that] wherein the species differentiation of pig DNA from various other species is made using the hybridization probe pair A1/A2 as the hybridization probe pair.

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37. (Amended) The method according to [claims 33 and 34] claim 33,  
[characterized in that] wherein the hybridization probes are used in combinations C1/C2;  
A1/B2; A1/A2; C1/A2; B1/B2; B1/A2 for the species differentiation between various  
species.

38. (Amended) LightCycler hybridization probes for exon 5, [characterized by]  
comprising the sequences:

A1: 5'- tgc ata ttt gtt tca tcc ggg caa att -fluorescein -3'

A2: 5'- LC Red 705 - tta aag gca caa gat ttc tat ggg ga - ph -3'

B1: 5'- tgc ata ttt att aca tcg ggg caa att -fluorescein -3'

B2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

C1: 5'-tgc ata ttt gtt aca tcg ggg taa att fluorescein -3'

C2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

39. (Amended) LightCycler hybridization probes for exon 6, [characterized by]  
comprising the sequences

PTENex6FL

5'- tca tct gga tta tag acc agt ggc act - fluorescein -3'

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PTENex6LC 640

5'- LC Red 640 - ttc aca aga tga tgt ttg aaa cta ttc caa- ph -3'

PTENex6F\*

5'- gtg cca ctg gtc tat aat cca gat- fluorescein -3'

PTENex6L\* 705

5'- LC Red 705- ttc ttt aac agg tag cta taa taa tac aca ta- ph -3'

40. (Amended) [The] LightCycler hybridization probes for exon 7,  
[characterized by] comprising the sequences

PTENex7F\*

5'- taa agg tga aga tat att cct cca att ca - fluorescein -3'

PTENex7L\*640

5'-LC Red 640- acc cac acg acg gga aga caa g - ph -3'

PTENex7 FL

5'-ggtaacggctgagggaactcaaagtac - fluorescein -3'



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PTENex7 LC (705-labeled)

5'-LC Red 705- tgaacttgcttcccgctcgtgtgg- ph -3'

41. (Amended) [The] LightCycler hybridization probes for exon 8,  
[characterized by] comprising the following sequences

PTENex8F\*

5'- tga caa gga ata tct agt act tac ttt aac aaa-fluorescein -3'

PPTENex8L\* 705

5'-LC Red 705 - ctt gac aaa gca aat aaa gac aaa gc- ph -3'

PTENex8 FLU

5' - tgctatcgatttcttgatcacatagacttccatttt - fluorescein -3'

PTENex8 LCR (640-labeled)

5'-LC Red 640- actttttctgaggtttcctctggtcctggtat - ph -3'

42. (Amended) [The] LightCycler hybridization probes for exon 9,  
[characterized by] comprising the following sequences

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PTENex9 FL

5'-aac atc tgg tgt tac aga agt tga act gct- fluorescein -3'

PTENex9 LC 640

5'-LC-640- cct ctg gat ttg acg gct cct cta ct - ph -3'

43. (Amended) Hybridization probe pair AI/A2: specific to PTEN pseudogene  
pig, [characterized by] comprising

SEQ No. 3 A1: 5'- tgc ata ttt gtt tca tcc ggg caa att -fluorescein -3'

SEQ No. 4 A2: 5'-LC Red 705- tta aag gca caa gat ttc tat ggg ga - ph -3'

44. (Amended) Hybridization probe pair B1/B2: specific to pseudogene man,  
[characterized by] comprising

SEQ No. 5 B1: 5'- tgc ata ttt att aca tcg ggg caa att -fluorescein -3'

SEQ No. 6 B2: 5'-LC Red 640- aag gca caa gag gcc cta gat ttc ta -ph -3'

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45. (Amended) Hybridization probe pair C1/C2: specific to PTEN pseudogene man (C2) and homologue of pig (C1) [characterized by] comprising

SEQ No. 7 - C1: 5'- tgc ata ttt gtt aca tcg ggg taa att - fluorescein -3'

SEQ No. 8 - C2: corresponds to probe B2.

47. (Amended) DNA sequences of homologues of the PTEN/MMAC1 gene and/or of homologues of the PTEN/MMAC1 pseudogene, which are compiled in the annex under "list of species sequences", which as compared to the PTEN/MMAC1 gene and/or the PTEN/MMAC1 pseudogene comprise genetic variants [such as] comprising base substitutions and/or insertions and/or deletions and are suited for identifying corresponding species.

48. (Amended) A kit for carrying out the method according to [claims 1 to 18 and further claims] claim 1, comprising:

- a) one or more vessels comprising PCR and/or sequencing oligonucleotides binding to highly conserved genes, the oligonucleotides being optionally labeled radioactively or by means of a dye or in another way,
- b) vessels having further common reagents for DNA amplification and/or DNA analysis, [in particular for DNA sequencing,]

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and

- c) a vessel containing a control DNA which is suited for testing the oligonucleotides and the reaction conditions.

49. (Amended) The kit according to claim 48 [for carrying out the method according to claims 1 to 18 and further claims], comprising:

- a) one or more vessels with PCR and/or sequencing oligonucleotides [according to claims 19 to 26].

50. (Amended) [Kit] ~~The kit~~ for identifying species for carrying out the method according to [claims 1 to 18 and further claims] claim 1, comprising:

- a) a vessel having an oligonucleotide pair comprising the following sequences:

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3' and 5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

- b) two vessels with one of the following sequencing oligonucleotides each, these oligonucleotides being optionally labeled radioactively or by means of a dye or in another way:

5'- cag gaa aca gct atg ac -3' and

5'- cga cgt tgt aaa acg acg gcc agt -3',

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- c) a vessel containing a control *DNA*, which is suited for testing the oligonucleotides and the reaction conditions.

51. (Amended) The kit (Light Cycler Kit) for carrying out the method according to [claims 32 to 37 and further claims] claim 32, comprising

- a) one or more vessels containing PCR primers and hybridization probes, which bind to highly conserved genes, the hybridization probes being optionally labeled by means of a dye,
  - b) vessels containing further common reagents for DNA amplification and/or DNA analysis, [in particular for the Light Cycler Analyses,]
- and
- c) a vessel containing a control DNA which is suited for testing the oligonucleotides and the reaction conditions.

52. (Amended) The kit (Light Cycler Kit) for carrying out the method according [to claims 32 to 37 and further claims] claim 32, comprising:

- a) one or more vessels with PCR primers and hybridization probes [according to claims 38 to 42].

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Method for Identifying Organisms by Means of Comparative  
Genetic Analysis and Primers and Hybridization Probes for  
Carrying out this Method

**Description**

The present invention relates to a method for the genetic analysis of organisms of different species of animal and/or plant by studying coding and non-coding areas of highly conserved genes or pseudogenes and their homologues with various species of animal and plant.

The known methods serving for simply, readily and precisely determining gene sequences to detect relationships and to identify organisms are based on the use of oligonucleotides which are specific to a species. These oligonucleotides are necessary to provide sufficient genetic material for the subsequent sequence reaction by means of polymerase chain reaction or other methods. An oligonucleotide is usually a short synthetically produced molecule which binds to a specific gene segment and has a specific sequence complementary to this strand. The drawback of methods based on the use of oligonucleotides consists in that gene sequences of different species usually differ strongly. In order to sequence organisms of different species, species-specific DNA sequences must, as a rule, be determined by means of methods which are costly and time-consuming, and the corresponding oligonucleotides which bind specifically to these DNA sequences must be synthesized in a second step. These two steps are usually required for each organism to be studied of unknown origin. For a subsequent analysis for determining the identity or the relationship due to the gene sequence the DNA of the organisms to be studied must then be tested with a usually large number of different oligonucleotides. Since usually only oligonucleotides of few

species are available, the analysis often fails to be a success in the case of rare species of animal. The analysis is also time-consuming and expensive.

Other methods which utilize e.g. restriction length polymorphisms of the organisms or methods used for amplifying a mixture of short oligonucleotides randomized as regards their sequence (random amplified polymorphic DNA, RAPD) do not result in an accurate and unambiguous analysis of the gene sequence of individual animals. As a result, it is difficult to determine the relationship between organisms by means of these methods.

It is thus the object of this invention to provide a method for the genetic analysis of organisms, which is highly sensitive, supplies reliable results while consuming little time, is also suited for major serial examinations and routine tests, and can optionally also be carried out automatically. The object of this invention also consists in developing means or products for carrying out this method. The invention is realized according to the claims, the subclaims being preferred variants.

The subject matter of the invention relates to a method by which the gene sequence of a gene area of various species can be determined readily, simply and safely in a reproducible way. Three preconditions must be met here: The oligonucleotides serving as primers for the amplification of the DNA must bind to areas of the genome which are highly conserved to ensure an amplification of genetic material by means of an identical oligonucleotide pair in all or the greatest possible number of different organisms. These oligonucleotides must cover an area which has a great sequence diversity between different species to enable a differentiation. The area which is covered by the oligonucleotides used as primers should be as small as possible to maximize the yields of amplicates and to ensure that copies can also be obtained from strongly degraded DNA in the starting material.

According to the invention this object is achieved by providing a method of determining the identity or the

relationship by comparing coding and non-coding areas of highly conserved genes of pseudogenes and homologues. As a result, it is ensured that a single oligonucleotide pair binds to DNA sequences highly conserved between various species and thus enables a gene segment identical for all species to be amplified. The oligonucleotides comprise one or more gene areas having the greatest possible sequence differences between different species. The determination of the gene sequence of this highly polymorphous gene area in a subsequent reaction step enables the gene sequence to be allocated to a specific species.

Genotyping is made by sequencing or by other methods which are suited for the detection of sequence variants. This comprises genotyping methods assisted by polymerase chain reaction (PCR), such as allele-specific PCR, other genotyping methods using oligonucleotides (e.g. "dot blotting", or "Oligonucleotide Ligation Assays" (OLA)), methods using restriction enzymes, analysis of length polymorphisms and "single nucleotide polymorphisms" (SNP), analysis by means of spectroscopic methods such as "matrix-assisted laser desorption/ionization mass spectroscopy" (MALDI), chromatographic methods such as DHPLC for separating DNA strands of differing lengths and sequences and in principle any method available at present or in the future for variant detection, including DNA, RNA and PNA hybridization methods, light cycler technology, TaqMan and molecular beacon technology and the chip technology in all its technological realizations.

The following steps are preferably carried out in the method according to the invention:

- a) DNA isolation: DNA is isolated and purified from blood samples, tissues, hair, foodstuffs and samples containing DNA.
- b) Polymerase chain reaction: The polymerase chain reaction serves for amplifying DNA for the subsequent sequencing reaction. In the polymerase chain reaction, one or more oligonucleotide pairs bind to the DNA to be analyzed (template DNA) of genes which are highly conserved between organisms of various species. In each case, one of the



molecules of an oligonucleotide pair (sense and antisense oligonucleotide) is complementary to one of the two template DNA strands at the 5' or 3' end of a DNA sequence. The binding is oriented such that the synthesis products obtained in an oligonucleotide-controlled polymerase chain reaction using one of the two oligonucleotides each may serve, following denaturation, as a matrix for binding the respectively other oligonucleotide. The oligonucleotide pair flanks the area which shall be copied. These oligonucleotides are extended in accordance with the nucleotide sequence of the template strand by means of polymerase and following the addition of nucleotide building blocks. Binding, extension and denaturation take place at different temperatures and are usually carried out 20 to 35 times in succession so as to multiply exponentially the area covered by the oligonucleotides.

c) Agarose gel electrophoresis: The DNA fragments are separated from the oligonucleotides in an agarose gel with a voltage being applied, and the band specific to the PCR product is excised using a scalpel and purified.

d) Sequencing reaction: Another polymerase is added as well as all nucleotide building blocks and special nucleotide building blocks which terminate the chain in the extending reaction. As a result, DNA fragments form which differ in length by one nucleotide each. Areas within the gene are sequenced which as regards their gene sequence are polymorphous between the different species. Therefore, the DNA sequence characteristic of a species can be determined and allocated to a species.

e) Polyacrylamide gel electrophoresis: If after concluding the sequencing reactions the differently long DNA strands are separated on a high-resolution polyacrylamide gel in an electric field, shorter DNA strands will migrate more rapidly than longer strands. In the pattern of bands forming, the order from shorter band to the respective band next in length - allocated to the corresponding bases A, C, G or T - corresponds to the complementary DNA sequence of the template. As a result, the sequence of the DNA strand, which was amplified by means of polymerase chain reaction

beforehand, becomes readable.

f) Comparative analysis of the gene sequence between the animal species and storage of the sequencing data.

For the amplification the method according to the invention preferably uses oligonucleotide pairs which bind to coding or non-coding areas of highly conserved genes or pseudogenes and their homologues, i.e. to genes or pseudogenes and their homologues which show no, or only minor, sequence differences between the individual species. This ensures that an amplificate can be formed in each gene segment to be analyzed of the most differing species by means of a primer pair.

The method according to the invention preferably serves for analyzing areas of the gene or pseudogene and their homologues, which differ as regards their gene sequence between organisms of different species. These may be sequences of coding or non-coding DNA.

The method according to the invention uses for certain batches for the polymerase chain reaction several differently long oligonucleotide pairs in a reaction mixture (multiplex PCR), all of which are partially identical with a starting sequence. Here, the sense or antisense oligonucleotides differ each as regards the nucleotide sequence such that the lengths of some oligonucleotides differ in the 3' region by one or more nucleotides. The use of several oligonucleotides differing in length shall ensure that binding of the oligonucleotides to the template DNA will be possible even if the template DNA differs from some oligonucleotides used as regards the 3' region. The correspondence in the 3' region of the oligonucleotides with the template DNA is essential for the specific amplification and should thus be as precise as possible in this area.

Example:

*sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat  
gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat  
-3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat t -  
3',

*antisense:*

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc t -3'.

In another preferred embodiment of the method according to the invention, multiple oligonucleotide pairs are used in a multiplex PCR, which have equal length but differ at one or more positions of the 3' end of the oligonucleotides as regards their nucleotide sequence. The use of a reaction mixture of several oligonucleotides differing as regards the base sequence at the 3' end of the oligonucleotide shall ensure that an amplificate can be formed in the most different species by means of a primer pair.

The method according to the invention preferably analyzes segments of the gene or pseudogene and their homologues which differ as regards their gene sequence between organisms of different species. These may be sequences of coding or non-coding DNA.

The method according to the invention uses for certain batches for the polymerase chain reaction several differently long oligonucleotide pairs in a reaction mixture (multiplex PCR), all of which are partially identical with a starting sequence. Here, the sense or antisense oligonucleotides differ each as regards the nucleotide sequence such that the lengths of some oligonucleotides differ in the 3' region by one or more nucleotides. The use of several oligonucleotides differing in length shall ensure that binding of the oligonucleotides to the template DNA will be possible even if the template DNA differs from some oligonucleotides used as regards the 3' region. The correspondence in the 3' region of the oligonucleotides with the template DNA is essential for the specific amplification and should thus be as precise as possible in this area.

*Example**sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat  
gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat

-3',

5' - cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat t

-3',

*antisense:*

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc t -3'.

In another preferred embodiment, the method according to the invention uses multiple oligonucleotide pairs in a multiplex PCR, which have equal length but differ at one or more positions of the 3' end of the oligonucleotides as regards the nucleotide sequence. The use of a reaction mixture of several oligonucleotides differing as regards the 3' end of the oligonucleotide shall ensure that the oligonucleotides are bound to the template DNA even if the template DNA differs at the 3' binding site of the oligonucleotide from the usually used oligonucleotide. For this purpose, a mixture of different oligonucleotides which have all conceivable nucleotide sequences at their 3' end is provided for the amplification.

*Example:*

*sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gaa -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gag -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gat -3',

*antisense:*

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt a -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt g -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt t -3'.

When suitable oligonucleotide sequences are selected, the method according to the invention pays attention to the fact that as many oligonucleotides as possible correspond at their 3' end with the first nucleotide of the codon which codes for

the highly conserved amino acid. It follows from theoretical considerations and also on the basis of observations that every second nucleotide has within a codon a degree of correspondence between organisms of differing species higher than that of the first or third nucleotide of the codon. Since the amplification will usually only function if along with other preconditions the nucleotide located at the 3' end of the oligonucleotide is exactly complementary to the opposite nucleotide of the template DNA strand - i.e. A faces T or G faces C -, the sequence of the oligonucleotides employed is chosen such that the last nucleotide at the 3' end of the oligonucleotide binds, if possible, to the most highly conserved nucleotide of the codon coding for an amino acid.

The method according to the invention preferably uses for different segments of the gene, of the pseudogenes and its homologues a single oligonucleotide pair each, which binds to the highly conserved tumor suppressor gene PTEN/MMAC1, its pseudogene and their homologues in different species, namely in the area of the gene which shows major correspondence between the species [Steck et al., 1997]. This area concerns the entire coding sequence of the gene, pseudogene and its homologues as well as exon-intron transitions and 5'- and 3'-untranslated regions of the gene, pseudogene and their homologues.

The areas amplified by means of the above described methods comprise sequence segments which have more or less great sequence differences between the individual species. This applies in particular to intron regions and specifically to intron 4. The oligonucleotide pair used for the amplification may bind to exons 4 and 5, since the PTEN/MMAC1 gene, pseudogene and their homologues show no, or only slight, differences between the species in exon 4 and exon 5. The intron region 4 covered by both oligonucleotides, which in contrast to the exon regions 4 and 5 between the species has considerably greater sequence differences, is amplified. Thereafter, part of this intron region is sequenced preferably by means of a sequencing reaction (see figure 1).

By means of comparative analyses, it is possible to determine due to the correspondence of intron sequences whether different samples belong to an identical species. It is also possible to determine the relationship of different species by means of comparative analysis of an intron region comprising only some to several hundred bases of the PTEN/MMAC1 gene and its homologues due to the similarity of the sequences. The general rule is that for the differentiation of closely related species the intron segments which must be studied have to be longer than those of distantly related species. This procedure described for exons 4/5 and intron 4 may also be applied to all of the other introns enclosed by exons. It also applies to pure exon regions, pseudogenes and the 5'- and 3'-untranslated regions as well as their homologues, in these cases the sequence differences between the individual species being less than in the intron regions.

In a preferred embodiment of the method according to the invention, highly conserved pseudogenes and their homologues are studied in various organisms and used for determining the species-specific gene sequence. These pseudogenes and their homologues have the advantage that they are also highly conserved as regards their nucleotide sequence and allow an amplification in certain species. However, the sequence differs in some areas of the pseudogene and its homologues between different species, so that pseudogenes and their homologues can be used for the species-specific characterization of organisms. Since the intron regions lack, they - like pure exon regions and the 5'- and 3'-untranslated regions - are suitable for the analysis, in particular of DNA degraded due to environmental influences, because of the small size.

An advantageous execution of the method utilizes the deletion identified by inventors and having a length of 9 base pairs in a PCR product (see figure 3 and Example 2), which corresponds to a gene sequence variant of PTEN/MMAC1 and was amplified from DNA of pig cells. This deletion having a

length of 9 base pairs is typically found in domestic pigs and all examined wild boars and as for the rest in no other examined species. In a variant of an embodiment of the invention this difference in length serves for proving pig meat in foodstuffs. This variant in length is genotyped by sequencing or by other methods suitable for detecting this deletion. They comprise PCR-assisted genotyping methods such as PCR by means of species-specific oligonucleotides, hybridization techniques such as the light cycler technology or other genotyping methods using restriction enzymes, and in principle any method available at present or in the future for detecting variants, including the chip technology and all of their technological realizations. Deletions and insertions, as found in different species in intron 4, exon 8 and in the 5'-untranslated region, can also be used correspondingly for identifying species (see annex: "List of species sequences").

The method according to the invention was made with DNA from different species as a model system and the gene sequence in the area of two segments in intron 4 of the PTEN/MMAC1 gene and its homologues was determined. It was possible to amplify all species with only one oligonucleotide pair. In the sequencing reaction with only one oligonucleotide and in the subsequent analytical polyacrylamide gel electrophoreses it turned out that all investigated species differ as regards the nucleotide sequence in the intron region (see annex: "List of species sequences"). Since the PTEN/MMAC1 tumor suppressor gene and its pseudogene and their homologues are conserved, the method can in other species also amplify successfully the corresponding gene sequences of lower organisms and possibly plants by means of an oligonucleotide pair. The method according to the invention is thus suited to determine the identity and relationship of various organisms. A data library was established which can be used for identifying different species and humans. It comprises PCR primers, sequencing primers and hybridization probes as well as sequences of the coding and non-coding areas including select highly variant intron regions, of exon regions, of the

5'-untranslated region of the gene and its homologues as well as of the pseudogene and its homologues from the most differing vertebrates (see annex: "List of species sequences"). On the one hand, the method according to the invention is suited to determine readily, simply and safely in a reproducible manner the relationship in certain species which are clearly classified (phylogenetic analyses). On the other hand, it is possible to determine by a comparison with gene sequences which can clearly be allocated to a species, the identity of tissue samples, blood samples and foodstuffs and all samples which contain DNA and are of unknown origin. Therefore, the method is also suited for applications in forensic medicine. Since in a preferred embodiment of the method according to the invention DNA is used as the starting material, organic samples (e.g. blood, saliva, tissue residues) can clearly be allocated to human or animal origin because of their gene sequence. Due to the possibility of comparing the collected DNA sequence with an already established data library with DNA sequences of known species it is possible to make statements on the species. If the gene sequence is unknown, it is possible due to sequence similarities to make statements on the relationship which the species to be studied has with a comparative DNA.

An advantageous embodiment of the invention utilizes hybridization probes for distinguishing the DNAs of various species among one another. This method uses differing ways for distinguishing the species on the basis of the LightCycler analysis system (company of Roche Molecular Biochemicals) using hybridization probes (patents WO 97/46714; WO 97/39008).

The LightCycler analysis system makes possible the amplification, detection and specific analysis of DNA of differing species and their differentiation within the shortest possible time and with moderate expenditure. The LightCycler is a micro-volume fluorimeter having a thermocycler combining rapid thermocycling with real-time fluorescence observation during the PCR (Wittwer et al., 1997a). By means of this technology, the time for the



In order to detect the specific amplification product and to differentiate differences in the target sequence, the LightCycler provides the possibility of carrying out melting point analyses. The melting point analysis is based on the fact that two complementary DNA strands are separated at a characteristic temperature, the melting temperature, into two individual strands. This melting temperature depends on the base composition in which sequences rich in GC have a higher melting point than sequences with predominantly AT bases. If a melting point analysis is carried out by removing more or less well complementary hybridization probes from the template DNA under certain temperature conditions, there is a melting profile characteristic of the fitting between probe and target sequence to be analyzed in the form of a fluorescence intensity measurement. As a result, the changes in the target sequence can be detected. If the sequences are fully complementary to one another, the probes will fully hybridize. If the target sequence contains changes in its base sequence, the melting point of the probes is lowered

Hybridization probes according to the invention were selected on the one hand with the target area of the 9-base pair deletion of the pig pseudogene. Probe A1/A2 enables a

distinction of the pig DNA from all of the other species.

Since by the above PCR primer pair, it is not only the pseudogene but also the gene area of exon 5 of the pig homologue of PTEN/MMAC1 that is amplified, another probe pair was provided with the complementary sequence of the pig homologue. This is probe C1/C2.

Since the different species in this gene area show minor sequence differences which should be used for a detailed differentiation among one another, a third probe pair which corresponds to the sequence of the human pseudogene in the selected area was constructed (probe B1/B2).

According to the invention, the probes A1/A2 are concerned: specific to PTEN pseudogene pig:

A1: 5'- tgc ata ttt gtt tca tcc ggg caa att - fluorescein -3'

A2: 5'- LC Red 705 - tta aag gca caa gat ttc tat ggg ga - ph -3'

Probes B1/B2: specific to PTEN pseudogene man:

B1: 5'- tgc ata ttt att aca tcg ggg caa att - fluorescein -3'

B2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

Probes C1/C2: specific to PTEN homologue pig:

C1: 5'- tgc ata ttt gtt aca tcg ggg taa att - fluorescein -3'

C2: corresponds to probe B2

The positions of probes A1, A2, B1, B2, C1 and C2 in exon 5 are shown in figure 4.

The separate use of these three probe pairs and the use of the probes in various possible combinations (1 donor dye + 1 acceptor dye) yields for each individual species a characteristic panel of differing melting points enabling a clear distinction between the species (see figures 5 and 7).

The use of these hybridization probe combinations also permits to carry out studies in reaction mixtures of two or more different species.

A parallel analysis/detection of the fluorescence of two different wavelengths (640 nm and 705 nm) is possible in a multiplex reaction with two different probe pairs such that the respective donor probes are labeled differently. The acceptor probes may be identical or differ as regards their sequence. Having concluded the melting point analysis, a melting point specific to the corresponding probe and the target sequence covered by it is obtained for each of the two wavelengths.

The alternative combination from two different donor probes and one acceptor probe of one wavelength yields for a reaction mixture of two different species having minor sequence differences in the target area two melting points within one wavelength.

In general, the following general multiplex reaction batches are possible which are characterized in that at least one hybridization probe pair is used and at least one gene segment is amplified, differing hybridization probe pairs hybridize to differing gene segments, and the melting points of the different combinations are determined and compiled for each species into a panel or used for the identification. These general multiplex reaction batches can also be characterized in that not only at least one hybridization probe pair is used and not only at least one gene segment is amplified but also DNA of at least one species is used and thus different hybridization probe pairs hybridize to differing gene segments of different species, and the melting points of the different combinations are determined and compiled for each species into a panel or used for the identification.

On this basis, the following analytical approaches are possible, melting point overlaps having to be avoided.

- a) Analysis of an unknown species sample with two different donor probes and one acceptor probe of one wavelength or of differing wavelengths,
- b) Analysis of two or more unknown species samples with two different donor probes and one acceptor probes of one wavelength or of different wavelengths,
- c) analysis of an unknown species sample with a mixture of two or more donor probes and acceptor probes each,
- d) analysis of a mixture of unknown species samples with one donor probe and one acceptor probe,
- e) analysis of a mixture of unknown species samples with two or more different donor probes and two or more acceptor probes of one or more different wavelengths.

This procedure can be applied to all highly conserved segments of the PTEN/MMAC1 gene, pseudogene and their homologues and also to other highly conserved genes when species shall be distinguished.

Select primers for the amplification are defined in claims 18 to 25 and select hybridization probe sequences for the LightCycler application are defined in claims 34 to 42 for different exons of the PTEN/MMAC1 gene, pseudogene and their homologues. Due to the high conservation of the gene in the evolution, primers and hybridization probes are also possible in all of the other conceivable areas of the gene, pseudogene and their homologues which can be used according to the above described principle for distinguishing species.

These probes and/or primers can be combined as desired in accordance with the multiplex principle with the aim of differentiating species.

The general rule is that all of the described methods and all methods conceivable at present and in the future for analyzing the DNA sequence variants can be applied to both the sense strand and antisense strand. This equivalence principle also applies to all PCR, sequence primer and hybridization probes which are described in this patent

application and the described gene sequences of the individual species of animal.

The following examples shall explain the invention in more detail.

**Example 1**

In this experiment, the sequence diversity between human DNA and elephant DNA is determined. The arrangement shown in figure 1 was chosen. The comparative sequence analysis was carried out by determining the species-specific sequences of intron 4 of the tumor suppressor gene PTEN/MMAC1 and its homologues. An oligonucleotide pair which is specific to areas within exons 4 and 5 of the DNA sequence of this gene for mice was chosen and subsequently synthesized (company of Amersham Pharmacia).

Oligonucleotide 1: 5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3'

Oligonucleotide 2: 5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3'

The oligonucleotides for the polymerase chain reaction were constructed such that they have one sequence each at their 5' end (underlined) which is complementary to the two oligonucleotide used for the sequencing reaction. As a result, it is ensured that the specific oligonucleotide parts bind in each case specifically to the template DNA (not underlined) but the oligonucleotides used for sequencing may differ from those used for the polymerase chain reaction, which adds quite generally to the quality of the sequencing reaction. The specific portion of the oligonucleotides binds in each case to exon 4 and exon 5 of the PTEN/MMAC1 tumor suppressor gene and its homologues.

A veterinarian provided 3 ml of residual blood of an African elephant resulting from a routine operation. The DNAs from elephant blood and from human blood were isolated by means of the QIAGEN kit (QIAGEN company) in accordance with the protocols from the manufacturer and stored at -20°C until they were used.

In order to generate sufficient amounts of DNA for the sequencing reaction, a polymerase chain reaction (PCR) was carried out. 3 sample batches were provided and the following

volumes and final concentrations or amounts of substrates and units of polymerase were used: reaction vessel 1: elephant DNA 1  $\mu$ l (50 ng); reaction vessel 2: human DNA 1  $\mu$ l (50 ng); reaction vessel 3: no DNA but 1  $\mu$ l distilled water instead (negative control). All sample batches were provided with the following: 14.35  $\mu$ l distilled water; dNTPs (Promega company): 4  $\mu$ l (200  $\mu$ M);  $MgCl_2$  (InViTek): 1  $\mu$ l (2 mM); 10 x buffer (InViTek) consisting of 160 mM  $(NH_4)_2SO_4$ , 500 mM Tris-HCl, pH 8.8, 0.1 % Tween 20: 2.5  $\mu$ l; oligonucleotides 1 and 2: 1  $\mu$ l (0.2  $\mu$ M); Taq polymerase (InViTek): 0.15  $\mu$ l (0.75 units). All in all, 35 cycles were carried out in a Perkin Elmer Thermocycler 9600, denaturing taking place at 94°C for 50 seconds each, the oligonucleotides binding to the DNA strands at 53°C for 50 second, and extension taking place at 72°C for 60 seconds (one second longer per cycle). Before the first cycle was started, denaturing was carried out at 94°C for 3 minutes, and a last extension phase of 10 minutes at 72°C was carried out after the last cycle.

In order to check the success of the PCR, to isolate the amplified DNA and check the negative control, an agarose gel electrophoresis was carried out: A 0.8 % agarose gel was produced by adding ethidium bromide, and 1 x TAE buffer was added. 13  $\mu$ l PCR product each was added by pipetting to prepared sample bags or pockets to the gel matrix by adding 2  $\mu$ l dye and a voltage of 100 V was applied. Electrophoresis was stopped after 20 to 30 minutes, the DNA bands were made visible under a U.V. lamp and thereafter excised using a scalpel. The purification of the PCR product was possible by placing the excised bands on MicroSpin columns (Amersham Pharmacia) and centrifugation at 1020 g for 10 minutes. The eluate was diluted depending on the strength of the band on the agarose gel with up to 30  $\mu$ l distilled water.

The purified PCR products were subjected to a cycle sequencing reaction using oligonucleotides having the sequence 5' - Cy-5- cag gaa aca gct atg ac -3'. The oligonucleotides were labeled at the 5' end with the dye Cy-5. 4 reaction vessels (A, C, G, T) were provided per PCR



product, and the following volumes and concentrations were chosen: 3  $\mu$ l PCR product each per A, C, G, T; 1  $\mu$ l each per A, C, G, T reagent (Amersham Pharmacia) consisting of Tris-HCl (pH 9.5),  $MgCl_2$ , Tween 20, Nonidet P-40, 2-mercaptoethanol, dATP, dCTP, 7-deaza-dGTP, dTTP, thermoresistant pyrophosphatase and thermo sequenase DNA polymerase, reaction vessels A, C, G and T containing correspondingly ddATP, ddCTG, ddGTP and ddTTP. Thereafter, 1  $\mu$ l oligonucleotide of the above mentioned sequence (0.5  $\mu$ M) is added. The following reaction conditions were chosen: All in all, 25 cycles were carried out in a Perkin Elmer Thermocycler 9600 (Perkin Elmer company), denaturing taking place at 94°C for 20 seconds each, the oligonucleotides binding to the amplificate at 54°C for 30 seconds, and the DNA strands being extended while raising the temperature to the denaturing temperature. Prior to the commencement of the first cycle, denaturing was carried out at 94°C for 3 minutes and 30 seconds, and the last extension phase of 5 minutes was carried out at 72°C after the last cycle.

In order to make visible the sequence, the products were separated on a polyacrylamide gel in an electric field. For this purpose, the automatic laser fluorescence detection system A.L.F.express from the company of Amersham Pharmacia was chosen. The gel matrix was composed of 16.8 g urea (Gibco BRL), 5.2 ml 50 % long ranger gel solution (FMC company) and 4 ml 10 x TBE (Gibco BRL), which were diluted using distilled water to give 40 ml. The gel was polymerized by adding 140  $\mu$ l 10 % APS (Merck company) and 20  $\mu$ l TEMED (Serva company). 5  $\mu$ l formamide loading dye were added to each reaction vessel A, C, G, T, and added by pipetting to the prepared sample pockets. The following electrophoresis conditions were chosen: 1000 V, 40 mA, 40 W.

Following the gel electrophoresis, the sequences were generated by the A.L.F.express system (Amersham Pharmacia) and could then be compared with one another. Figure 2 shows the determined nucleotide sequence differences. A comparison of the sequences between elephant DNA and human DNA in the exon region yielded a great correspondence and served as a

control for the PTEN/MMAC1 specificity, whereas the intron region differed greatly.

#### **Example 2**

In this experiment, the sequence of a pig liver provided by a butcher's is compared with already available DNA of a pig. Beef salami served as a control. DNAs were obtained from 50 to 60 mg pig liver and 50 to 60 mg beef salami using the QIAGEN kit (QIAGEN) and purified. For the comparative analysis, the procedure carried out in Example 1 was chosen using oligonucleotides 1 and 2 for the polymerase chain reaction. A band having a length of about 300 bp was excised from the agarose gel for the sequence analysis. This band corresponds in length to the PTEN/MMAC1 pseudogene. After purifying the excised amplificate, Cy-5-labeled oligonucleotides with the sequence 5'- Cy-5- cag gaa aca gct atg ac - 3' were sequenced. The comparative analysis of pig liver from a butcher's with pig DNA already sequenced in this area resulted in a full correspondence. The control DNA (beef salami) has a sequence markedly differing from the pig DNA (see figure 3).

#### **Example 3**

The PCR and sequencing of exons 1 to 9 is carried out with the primers described in claims 19 to 26 with otherwise analogous reaction control. The corresponding sequences of the studied species are listed in the annex under "List of species sequences".

#### **Example 4**

This experiment is to show the species differentiation between pig DNA and human DNA using various combinations of hybridization probe pairs. The comparative analysis was made by determining the melting points of the hybridization probe pairs C1+B2, A1+B2, A1+A2, and C1+A2 in pig DNA and human DNA. The probes were chosen correspondingly and synthesized

(Tib Mol Biol, Berlin.

Probes:

A1: 5'- tgc ata ttt gtt tca tcc ggg caa att - fluorescein  
-3'

A2: 5'- LC Red 705 - tta aag gca caa gat ttc tat ggg ga - ph  
- 3'

B1: 5'- tgc ata ttt att aca tcg ggg caa att - fluorescein -  
3'

B2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph  
-3'

C1: 5'- tgc ata ttt gtt aca tcg ggg taa att - fluorescein -  
-3'

C2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph  
-3'

A precondition for a probe hybridization is the amplification of the target area with a suitable primer pair. For this purpose, the following primer pair was chosen and synthesized:

Sense primer: PTEN se 5'- atc ttg acc aat ggc taa gtg -3'  
(Tib Mol Biol)

Antisense primer: Zoo44aRV 5'- ttgt ctc tgg tcc tta ctt c-3'  
(Amersham Pharmacia)

Both primers bind to areas of the exon 5 of the PTEN/MMAC1 gene, pseudogene and their homologues, which comprises the target regions of about 172 base pairs.

The DNAs were isolated by means of the Quiagen kit (QIAGEN) from the blood of pigs and humans in accordance with the protocols of the manufacturer and stored at -20°C until they were used.

The real time PCR with hybridization probes and subsequent melting point analysis was carried out as follows: 3 rows with 4 sample batches each (glass capillary cuvette (Roche)) were provided in a cooled pipetting block (Roche). In row 1,

2  $\mu$ l (50 ng) pig DNA are supplied in each batch, 2  $\mu$ l (50 ng) human DNA are supplied to each batch of the 2<sup>nd</sup> row, and 2  $\mu$ l distilled water are supplied in row 3 for the negative controls. The hybridization probes were used for the corresponding batches in the following combinations: 1<sup>st</sup> batch of each row: A1, 1  $\mu$ l (0.1  $\mu$ M) and A2, 2  $\mu$ l (0.2  $\mu$ M); 2<sup>nd</sup> batch of each row: C1, 1  $\mu$ l (0.1  $\mu$ M) and B2, 2  $\mu$ l (0.2  $\mu$ M); 3<sup>rd</sup> batch of each row: A1, 1  $\mu$ l (0.1  $\mu$ M) and B2, 2  $\mu$ l (0.2  $\mu$ M); 4<sup>th</sup> batch of each row: C1, 1  $\mu$ l (0.1  $\mu$ M) and A2, 2  $\mu$ l (0.2  $\mu$ M).

The following volumes and final concentrations or amounts of substrates and units of polymerase were used in all sample batches: oligonucleotides PTEN se and Zoo44aRV 2  $\mu$ l (10  $\mu$ M) each; MgCl<sub>2</sub> (Roche Molecular Diagnostics): 2.4  $\mu$ l (4 mM); LightCycler DNA Master Hybridization Probes (Roche Molecular Diagnostics): 2  $\mu$ l of a stock solution concentrated by 10 times from Taq DNA polymerase, reaction buffer, dNTP mixture and 10 mM MgCl<sub>2</sub>. The final MgCl<sub>2</sub> concentration in the total reaction batch of 20  $\mu$ l is 5 mM. After fully loading all reagents, the glass capillary cuvettes are closed, centrifuged at 2000 g for 1 minute and inserted in the reaction carousel of the LightCycler provided for the capillaries.

All in all, 45 cycles are carried out in the LightCycler analysis system as follows: denaturation at 95°C for 1 second, binding of the oligonucleotides and probes at 54°C for 10 seconds and extension of the DNA strands at 72°C for 5 seconds. Denaturation was carried out at 95°C for 30 seconds before the first cycle started. The preliminary heating rate is programmed from denaturation to binding to 20°C/second, from binding to the extension of the DNA strands to 20°C/second and for the step of extension up to the denaturation to 20°C/second. The fluorescence resulting from the FRET of the probes binding complementarily side by side, is measured for observing the PCR at the end of the binding phase in each cycle. Probes which cannot bind fully to the target DNA due to sequence differences, since their binding temperature is below that of the oligonucleotides, cannot yet

be detected at that stage of fluorescence measurement.

After the complete amplification, a melting curve was finally recorded as follows: denaturation of the amplification products at 95°C for 5 seconds, cooling to 30°C with a preliminary heating rate of 20°C/second, holding of this temperature for 15 seconds and subsequent slow heating of 0.2°C/second up to 95°C. The fluorescence of the bound hybridization probes is recorded continuously up to the respective melting/dissociation during the slow temperature increase. A melting curve is recorded for each sample by recording the fluorescence signal as a function of the temperature. By forming the first negative derivative of the fluorescence against the temperature, the melting curves are converted into melting peaks.

After balancing the fluorescence signals at 640 nm and 705 nm against the standard curves prepared with a colorcompensation kit (Roche Molecular Diagnostics) for the respective wavelength, the melting points of pig DNA could be compared with those of the human DNA for the various probe combinations. In this connection, 2 melting points each could be recorded for the pig DNA for each probe pair, and one could be recorded each for the pseudogene and one for the gene. All melting points of the pig DNA differed from those of the human DNA (figure 5).

#### **Example 5**

In this experiment, the species differentiation of pig DNA from various other species of animal is shown by means of a single hybridization probe pair. For this purpose, DNAs from cattle, sheep, dwarf goat, chicken and turkey were used for the analysis (gene sequences in the annex "list of species sequences" under "sequences intron 4 exon 5").

The comparative analysis was carried out by determining the melting points of a hybridization probe pair whose sequences are specific to the pseudogene of pigs in the area of the 9 base pair deletion (A1/A2) and which was already used in Example 4. The primers used in Example 4 were employed for

the amplification. The DNA was collected from the bloods of said species by means of the QIAGEN kit (QIAGEN) and purified. For the real-time PCR a sample batch having 2  $\mu$ l (50 ng) of the corresponding DNA each is supplied for each species and a sample batch with 2  $\mu$ l distilled water is supplied for a negative control. Furthermore, 1  $\mu$ l (1  $\mu$ M) of the probe A1 and 2  $\mu$ l (0.2  $\mu$ M) of probe A2 were added by pipetting to each sample batch. To pipette further reagents the procedure of Example 1 was chosen as regards their volumes and concentrations and for the further reaction steps at the LightCycler.

A comparison of the melting points of pig DNA with those of the other animal species shows a clear distinction between pig DNA and those of other animal species (figure 6).

#### **Example 6**

In this experiment, the species differentiation between various animal species is shown by way of the following species with hybridization probes of Example 4:

Pig, deer, dog, Indian elephant, trout, quail, duck, goitred gazelle, mouse, guinea pig (gene sequences in the annex "list of species sequences" under "sequences intron4 exon5")

A comparative analysis was carried out by determining the melting points from various combinations of the hybridization probes from Example 4 in the combinations C1+B2, A1+B2, A1+A2, C1+A2, B1+B2 and B1+A2 and compiling a panel for each species of animal.

The DNA was obtained from the bloods of said species using the QIAGEN kit (QIAGEN) and purified. The primers listed in Example 4 were used for the amplification. For the further course of the experiment and the subsequent melting point analysis, the procedure in Example 4 was chosen, complemented by the additional probe combinations and greater numbers of species.

A panel of their melting points was compiled for the

corresponding probe combinations for each species (figure 7). A comparison of the panels of the different species yields a difference of each species from the others results as regards at least one melting point.

Figure 8 shows the mean value curves which were determined for selected probe combinations (C1+C2/A1+A2/B1+A2 and A1+C2 from 15 pig DNAs each and C1+C2/A1+A2/B1+A2 and C1+A2 from 15 human DNAs each) with the corresponding standard deviations. The experiment control corresponds to Example 4.

Furthermore, the sequences of the investigated species for exons 1-9 and the 5'-untranslated region of the PTEN/MMAC1 gene, pseudogene and their homologues are listed in the annex under "list of species sequences".

These examples for various probe combination panels can be enlarged by any number of probes and primers for the described gene PTEN/MMAC1, pseudogene and their homologues, but also for all of the other genes, and applied. A linear increase of the primers and probe combinations in the entire genome yields an exponential growth of differentiation possibilities of the most varying species among one another. By using sequence-specific probes and several and/or any number of probe combinations, a species can be distinguished from all of the other species by its very specific melting point panel.

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## **Annex**

### **List - figures 1 - 8:**

Figure 1: diagram of the method for identifying organisms according to Example 1

Figure 3: DNA sequence comparison of a pig with purchased pig liver and beef salami according to Example 2. The differences between the nucleotide sequences are shown. The 9-base pair long deletion (nucleotides 216-224) is striking in pig / pig liver.

Figure 5: Example 4 - melting point panels of pig and man

Figure 7: Example 6 - melting point panel of various species

Figure 8: standard deviations of select probes for pig (HS) and man (WT)

### Claims

1. A method of identifying organisms by comparative genetic analysis, characterized in that coding and/or non-coding areas and/or functionally significant areas of highly conserved genes and/or their homologous genes and/or their cDNA copies and/or their pseudogenes are amplified using PCR and are subsequently genotyped and analyzed.
2. The method according to claim 1, characterized in that one primer pair each is used for each specific segment of the highly conserved gene, which is located in the highly conserved exon region and/or non-coding areas and/or functionally significant areas and/or in the 5'- or 3'-untranslated area of the gene and binds in as many studied species DNAs as possible, preferable in all studied species DNAs, and enables the amplification of the corresponding gene area.
3. The method according to claims 1 and 2, characterized in that coding and/or non-coding areas located between the primers and being either highly variant intron regions and/or variant exon regions or 5'- or 3'-untranslated areas of the gene, are analyzed as regards their sequence and identified by comparison with the species-specific sequence variants.
4. The method according to claims 1 to 3, characterized in that either the sense strand or the antisense strand of any species DNA or also their PCR copies are used for the identification.
5. The method according to claims 1 to 4, characterized in that preferably animals are identified.
6. The method according to claims 1 to 4, characterized in that preferably vertebrates are identified.
7. The method according to claims 1 to 4, characterized in

that preferably mammals are identified.

8. The method according to claims 1 to 4, characterized in that preferably plants are identified.
9. The method according to claims 1 to 4, characterized in that genotyping is carried out by DNA sequencing, any hybridization methods, restriction fragment length analyses, chromatographic methods, spectroscopic and in particular mass-spectroscopic methods, allele-specific PCR or by other methods suitable for detecting DNA sequence variants.
10. The method according to claims 1 to 4, characterized in that exon and/or intron areas as well as functionally significant areas of the highly conserved tumor suppressor gene PTEN/MMAC1 and its homologues are used for amplification and subsequent genetic analysis.
11. The method according to claims 1 to 4, wherein cDNA copies of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.
12. The method according to claims 1 to 4, wherein pseudogenes or segments of pseudogenes of the PTEN/MMAC1 gene and its homologues are used for the genetic analysis.
13. The method according to claims 1 to 4, characterized in that preferably exons arranged side by side of the PTEN/MMAC1 gene and its homologues and/or the parts of the introns following the exons are analyzed genetically.
14. The method according to claims 1 to 4, characterized in that the exon regions 1 and 2 and/or 3 and 4 and/or 4 and 5 and/or 5 and 6 and/or 6 and 7 and/or 7 and 8 and/or 8 and 9 with the enclosed intron regions 1 and/or 2 and/or 3 and/or 4 and/or 5 and/or 6 and/or 7 and/or 8

as well as the 5'- and 3'-untranslated regions of the PTEN/MMAC1 gene and their homologues are used for the genetic analysis.

15. The method according to claims 1 to 4, characterized by selecting areas of highly conserved genes and/or pseudogenes and their homologues, constructing suitable oligonucleotides as primers which bind to the corresponding complementary coding and/or non-coding areas and/or functionally significant areas, amplifying them by means of a suitable technique and comparatively analyzing the sequence of the corresponding coding and/or non-coding area of various species by genetic analysis.
16. The method according to claim 15, characterized in that areas of the PTEN/MMAC1 gene and/or the pseudogene and their homologues are selected.
17. The method according to claims 15 and 16, characterized in that differing sequence segments of each individual exon, intron or untranslated region of the PTEN/MMAC1 gene and their homologues or the corresponding cDNA are selected.
18. The method according to claims 1 to 17, characterized in that genotyping of pig DNA which is obtained preferably from foodstuffs, is carried out on the basis of the gene sequence variant of PTEN/MMAC1 containing a 9-base pair long deletion.
19. An oligonucleotide primer for the PCR and the sequencing of exon 1 and/or 5'-untranslated region of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex1-401 sense

5'-cccttctactgcctcca -3'

PTENex1 -465 sense

5'- gggaggggggtctgagt -3'

PTENex1 ATG sense

5'- atgacagccatcatcaaaga -3'

PTENex1 R antisense

5'- aggtcaagtctaagtcgaatc -3'

20. The oligonucleotide primer for PCR and the sequencing of exon 2 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex2F sense

5'- atatttatccaaacattattgctat -3'

PTENex2R antisense

5'- cttactacatcatcaatattgttcc -3'

21. The oligonucleotide primer for PCR and the sequencing of exon 4, intron 4 and exon 5 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

Zoo43sUV sense

5'- tgtgctgagagacattatgac -3'

SPL5 sense

5'- aaattttaattgcagaggt -3'

Zoo44aRV antisense

5'- ttgtctctgggtcettacttc -3'

22. The oligonucleotide primer for PCR and the sequencing of exon 5 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTEN se sense

5'- atcttgaccaatgggctaagtg -3'

Zoo44aRV antisense

5'- ttgtctctggtccttacttc -3'

23. The oligonucleotide primer for PCR and the sequencing of exon 6 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex6F sense

5'- gga gta act att ccc agt cag ag -3'

PTENex6R antisense

5'- gca agt tcc gcc act gaa -3'

24. The oligonucleotide primer for PCR and the sequencing of exon 7 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex7F sense

5'- cct cag ttt gtg gtc tgc ca -3'

PTENex7R antisense

5'- c ctt ttt tag cat ctt gtt ctg ttt -3'

25. The oligonucleotide primer for PCR and the sequencing of exon 8 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex8F sense

5'- caa aat gtt tca ctt ttg ggt aaa -3'

PTENex8R antisense

5'- taa aat ttg gag aaa agt atc ggt t -3'

26. The oligonucleotide primer for PCR and the sequencing of exon 9 of the PTEN/MMAC1 gene and its homologues, characterized by the following sequences:

PTENex9F sense

5'- gtg aag ctg tac ttc aca aaa ac -3'

PTENex9tga antisense

5'- aaa aaa att cag act ttt gta att tg -3'

27. The method according to claims 1 to 17, characterized in that for the DNA amplification a mixture of oligonucleotides is used which differ at the 3' region of the oligonucleotide as regards its length by one or more nucleotides or which differ as regards their nucleotide sequence at the 3' end of the oligonucleotide at one or more positions.
28. The method according to claims 1 to 17 and 26, wherein the oligonucleotides

*sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat t -3',

*antisense:*

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta c -3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc t. -3'

are used for the amplification.

29. The method according to claims 1 to 17 and 26, wherein the oligonucleotides

*sense:*

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gaa -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat tat gac -3',



5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat  
tat gag -3',

5'- cga cgt tgt aaa acg acg gcc agt tgt gct gag aga cat  
tat gat -3',

*antisense:*

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt a  
-3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt c  
-3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt g  
-3',

5'- cag gaa aca gct atg act tgt ctc tgg tcc tta ctt t  
-3'

are used for the amplification.

30. The method according to claims 1 to 17, characterized in that DNA sequencing methods are used for the genetic analysis.
31. The method according to claims 1 to 17, characterized in that DNA sequencing techniques are used in the genetic analysis for the PTEN/MMAC1 and/or its pseudogenes and their homologues.
32. The method of distinguishing the DNA of various species, characterized in that at least one hybridization probe pair is used, the melting points of different combinations are determined and compiled for each species into a panel.
33. The method of distinguishing the DNA of various species, characterized in that at least one hybridization probe pair is used and at least one gene segment is amplified, differing hybridization probe pairs hybridize to different gene segments, and the melting points of the different combinations are determined and compiled for each species into a panel and/or compared with this panel for the purpose of identification.

34. The method of distinguishing the DNA of different species according to claim 33, characterized in that at least one hybridization probe pair is used and at least one gene segment of at least one species is amplified, differing hybridization probe pairs hybridize to different gene segments of various species, and the melting points of the different combinations are determined and compiled for each species into a panel and/or compared with this panel for the purpose of identification.
35. The method of distinguishing the DNA of various species according to claims 33 and 34, characterized in that at least two hybridization probes of SEQ Nos. 3 to 8 are used, the melting points of different combinations are determined and compiled for each species into a panel.
36. The method according to claims 33 and 34, characterized in that the species differentiation of pig DNA from various other species is made using the hybridization probe pair A1/A2 as the hybridization probe pair.
37. The method according to claims 33 and 34, characterized in that the hybridization probes are used in combinations C1/C2; A1/B2; A1/A2; C1/A2; B1/B2; B1/A2 for the species differentiation between various species.
38. LightCycler hybridization probes for exon 5, characterized by the sequences:
- A1: 5'- tgc ata ttt gtt tca tcc ggg caa att - fluorescein -3'
- A2: 5'- LC Red 705 - tta aag gca caa gat ttc tat ggg ga - ph -3'
- B1: 5'- tgc ata ttt att aca tcg ggg caa att - fluorescein -3'
- B2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta - ph -3'

C1: 5'- tgc ata ttt gtt aca tcg ggg taa att -  
fluorescein -3'

C2: 5'- LC Red 640 - aag gca caa gag gcc cta gat ttc ta  
- ph -3'

39. LightCycler hybridization probes for exon 6,  
characterized by the sequences

PTENex6FL

5'- tca tct gga tta tag acc agt ggc act - fluorescein  
-3'

PTENex6LC 640

5'- LC Red 640 - ttc aca aga tga tgt ttg aaa cta ttc  
caa- ph -3'

PTENex6F\*

5'- gtg cca ctg gtc tat aat cca gat- fluorescein -3'

PTENex6L\* 705

5'- LC Red 705- ttc ttt aac agg tag cta taa taa tac aca  
ta- ph -3'

40. The LightCycler hybridization probes for exon 7,  
characterized by the sequences

PTENex7F\*

5'- taa agg tga aga tat att cct cca att ca - fluorescein  
-3'

PTENex7L\*640

5'-LC Red 640- acc cac acg acg gga aga caa g - ph -3'

PTENex7 FL

5'-ggtaacggctgaggggaactcaaagtac - fluorescein -3'

PTENex7 LC (705-labeled)

5'-LC Red 705- tgaacttgtcttcccgtcgtgtgg- ph -3'

41. The LightCycler hybridization probes for exon 8, characterized by the following sequences

PTENex8F\*

5'- tga caa gga ata tct agt act tac ttt aac aaa-  
fluorescein -3'

PPTENex8L\* 705

5'-LC Red 705 - ctt gac aaa gca aat aaa gac aaa gc- ph -  
3'

PTENex8 FLU

5'- tgctatcgatttcttgatcacatagacttccatttt - fluorescein -  
3'

PTENex8 LCR (640-labeled)

5'-LC Red 640- actttttctgaggtttcctctggtcctggtat - ph -3'

42. The LightCycler hybridization probes for exon 9, characterized by the following sequences

PTENex9 FL

5'-aac atc tgg tgt tac aga agt tga act gct- fluorescein  
-3'

PTENex9 LC 640

5'-LC-640- cct ctg gat ttg acg gct cct cta ct - ph -3'

43. Hybridization probe pair A1/A2: specific to PTEN pseudogene pig, characterized by

SEQ No. 3 A1: 5'- tgc ata ttt gtt tca tcc ggg caa att -  
fluorescein -3'

SEQ No. 4 A2: 5'-LC Red 705- tta aag gca caa gat ttc tat  
ggg ga - ph -3'

44. Hybridization probe pair B1/B2: specific to pseudogene man, characterized by

SEQ No. 5 B1: 5'- tgc ata ttt att aca tcg ggg caa att -  
fluorescein -3'

SEQ No. 6 B2: 5'-LC Red 640- aag gca caa gag gcc cta gat  
ttc ta -ph -3'

45. Hybridization probe pair C1/C2: specific to PTEN  
pseudogene man (C2) and homologue of pig (C1),  
characterized by

SEQ No. 7 - C1: 5'- tgc ata ttt gtt aca tcg ggg taa att  
- fluorescein -3'

SEQ No. 8 - C2: corresponds to probe B2.

46. DNA sequences and/or fragments of homologues of the  
PTEN/MMAC1 gene and/or of the homologues of the  
PTEN/MMAC1 pseudogene, which code for proteins involved  
in the cell-cell adhesion and cell cycle regulation and  
have an indispensable function in embryogenesis of the  
respective species and which are compiled in the annex  
under "List of species sequences".
47. DNA sequences of homologues of the PTEN/MMAC1 gene  
and/or of homologues of the PTEN/MMAC1 pseudogene, which  
are compiled in the annex under "list of species  
sequences", which as compared to the PTEN/MMAC1 gene  
and/or the PTEN/MMAC1 pseudogene comprise genetic  
variants such as base substitutions and/or insertions  
and/or deletions and are suited for identifying  
corresponding species.
48. A kit for carrying out the method according to claims 1  
to 18 and further claims, comprising:
- a) one or more vessels comprising PCR and/or  
sequencing oligonucleotides binding to highly  
conserved genes, the oligonucleotides being  
optionally labeled radioactively or by means of a



b) vessels containing further common reagents for DNA amplification and/or DNA analysis, in particular for the Light Cycler Analyses,

c) a vessel containing a control DNA which is suited for testing the oligonucleotides and the reaction conditions.

a) one or more vessels with PCR primers and hybridization probes according to claims 38 to 42.

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(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES  
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(72) Erfinder; und

(75) Erfinder/Anmelder (*nur für US*): KOUFAKI, Olga, Niki

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— mit internationalem Recherchenbericht

[Fortsetzung auf der nächsten Seite]

(54) Title: METHOD FOR IDENTIFYING ORGANISMS BY MEANS OF COMPARATIVE GENETIC ANALYSIS AND PRIMERS AND HYBRIDISATION PROBES FOR CARRYING OUT THIS METHOD

(54) Bezeichnung: VERFAHREN ZUR IDENTIFIZIERUNG VON ORGANISMEN DURCH VERGLEICHENDE GENETISCHE ANALYSE SOWIE PRIMER UND HYBRIDISATIONSSONDEN ZUR DURCHFÜHRUNG DES VERFAHRENS

(57) Abstract: The invention comprises methods, primers and hybridisation probes for identifying organisms by means of comparative genetic analysis and is characterised in that coding and non-coding areas and/or functionally significant areas of highly conserved genes, pseudogenes or homologues are amplified using the PCR and then genotyped and analysed. The comparison of coding and non-coding areas of highly conserved genes, pseudogenes or homologues ensures that a single oligonucleotide pair bonds to DNA sequences that are highly conserved between different species, hereby allowing the amplification of a gene segment that is identical for all of the species. The oligonucleotides include one or more gene areas with the greatest possible sequence differences between different species. The determination of the gene sequence of this highly polymorphous area in a subsequent reaction step enables the gene sequence to be allocated to a specific species. In particularly preferred variants of embodiments of the invention, oligonucleotide pairs are found that make it possible to amplify the highly conserved tumour suppressor gene PTEN/MMAC1, its pseudogene and their homologues.

(57) Zusammenfassung: Die Erfindung umfaßt Verfahren, Primer und Hybridisationssonden zur Identifizierung von Organismen durch vergleichende genetische Analyse, dadurch gekennzeichnet, daß man kodierende, nichtkodierende Bereiche und/oder funktionell bedeutende Bereiche von hochkonservierten Genen, Pseudogenen oder Homologen mit Hilfe der PCR amplifiziert und nachfolgend genotypisiert und analysiert. Der Vergleich von kodierenden und nicht kodierenden Bereichen von hochkonservierten Genen, Pseudogenen oder Homologen gewährleistet, daß ein einziges Oligonukleotidpaar an DNA-Sequenzen, die zwischen verschiedenen Spezies hochgradig konserviert sind, bindet und damit die Amplifikation eines für alle Spezies identischen Genabschnitts erlaubt. Die Oligonukleotide schließen einen oder mehrere Genbereiche ein, die zwischen verschiedenen Spezies möglichst große Sequenzunterschiede aufweisen. Die Bestimmung der Gensequenz dieses hochgradig polymorphen Bereichs in einem darauf folgenden Reaktionsschritt erlaubt es, die Gensequenz einer spezifischen Spezies zuzuordnen. In besonders bevorzugten Ausführungsvarianten der Erfindung wurden Oligonukleotidpaare gefunden, die die Amplifikation des hochkonservierten Tumorsuppressorgens PTEN/MMAC1, seines Pseudogens und ihrer Homologen ermöglicht.

WO 00/55361 A3



Figure 1:

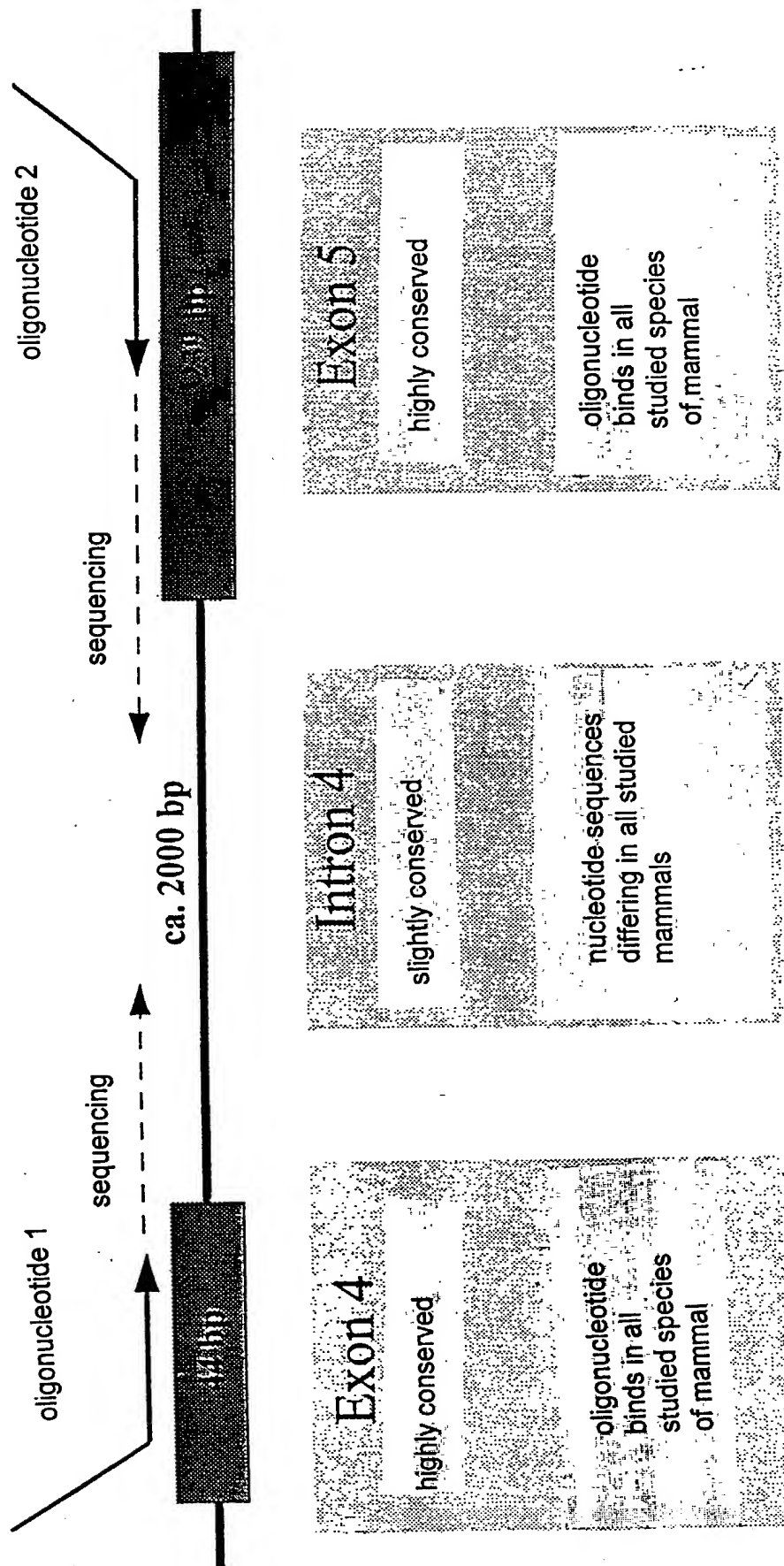
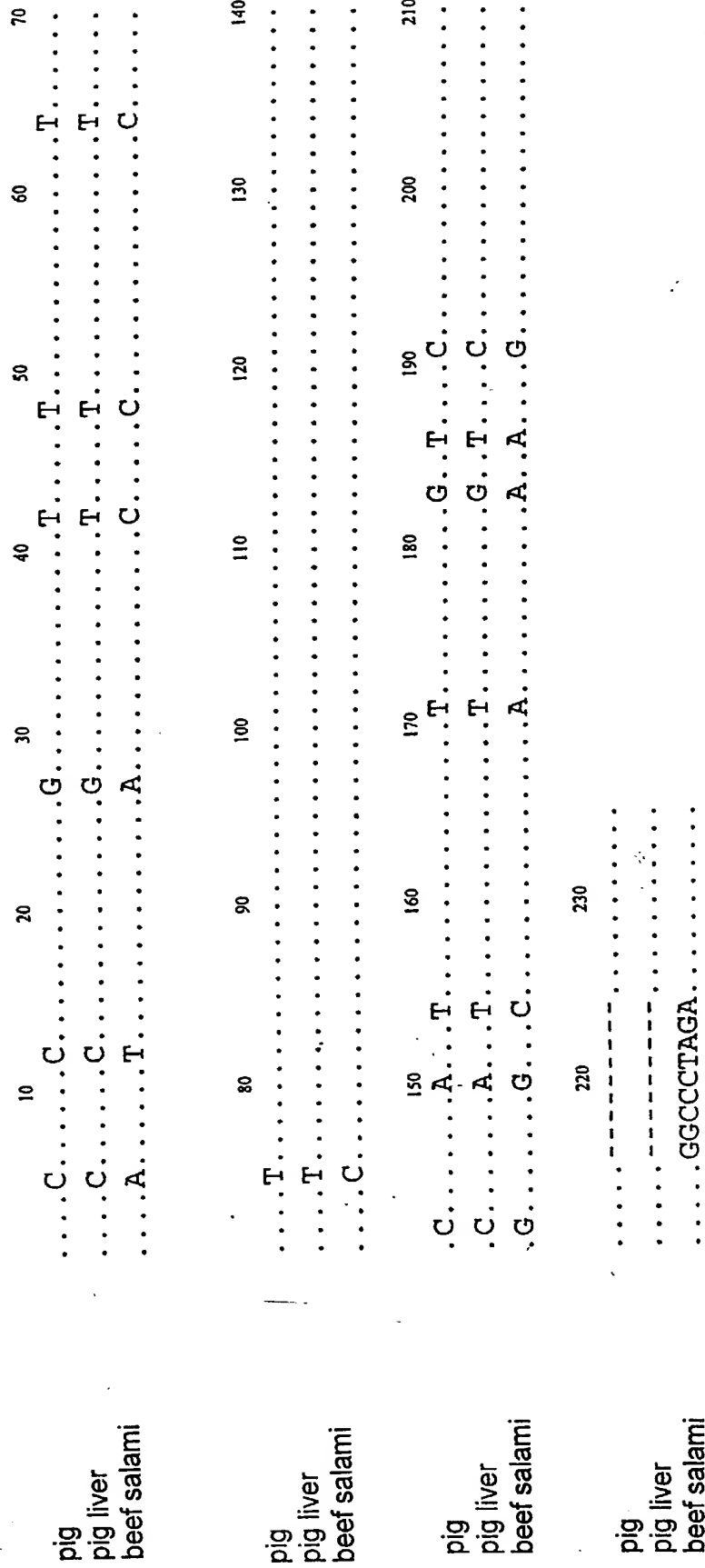


Figure 2

man	10	20	30	40	50	60	70
African elephant	T..C..T..A.....	..C...A.....	CTCT..A.CC..-G-..C-..				
	..C..A..C..C.....	TC..G.....	TAAG...G.TT..C.TA.C..T.A.....				
man	80	90	100	110	120	130	140
African elephant	T.....T.T.....	G.A.....G.A.....	CA.....G.....				TT
	C.,.....	A.G.....	TG.....G.....				GG....A-
man	150	160	170	180	190	200	210
African elephant	.....G.....	.....A.....	.....T.....				
	.....T.....	.....G.....	.....C.....				
man	220	230	240	250	260	270	280
African elephant	.....A.....	.....G.....	.....A.....				
	.....G.....	.....T.....	.....G.....				
man	290	300	310	320	330	340	350
African elephant	.....A.....	.....A.....	.....T.....				
	.....G.....	.....G.....	.....G.....				

Figure 3



**Figure 4:** Positions of the hybridization probes in exon 5 of the PTEN gene, pseudogene and their homologues

	150	B1	B2	220
man pseudogene	ATATG TGCATATTTTATTACATCGGGGCAAATT TTTA AAGGCACAAGAGGCCCTAGATTTCTA TGGGGAAGT			
		C1	C2	
pig gene	ATTIG TGCATATTTGTTTACATCGGGGTAATT TTTA AAGGCACAAGAGGCCCTAGATTTCTA TGGGGAAGT			
		A1	A2	
pig pseudogene	ATTIG TGCATATTTGTTTACATCGGGGCAAATT TTTAAGGCACAAGA ----- TTTCTATGGGGA AGT			

Figure 5: Melting point panel

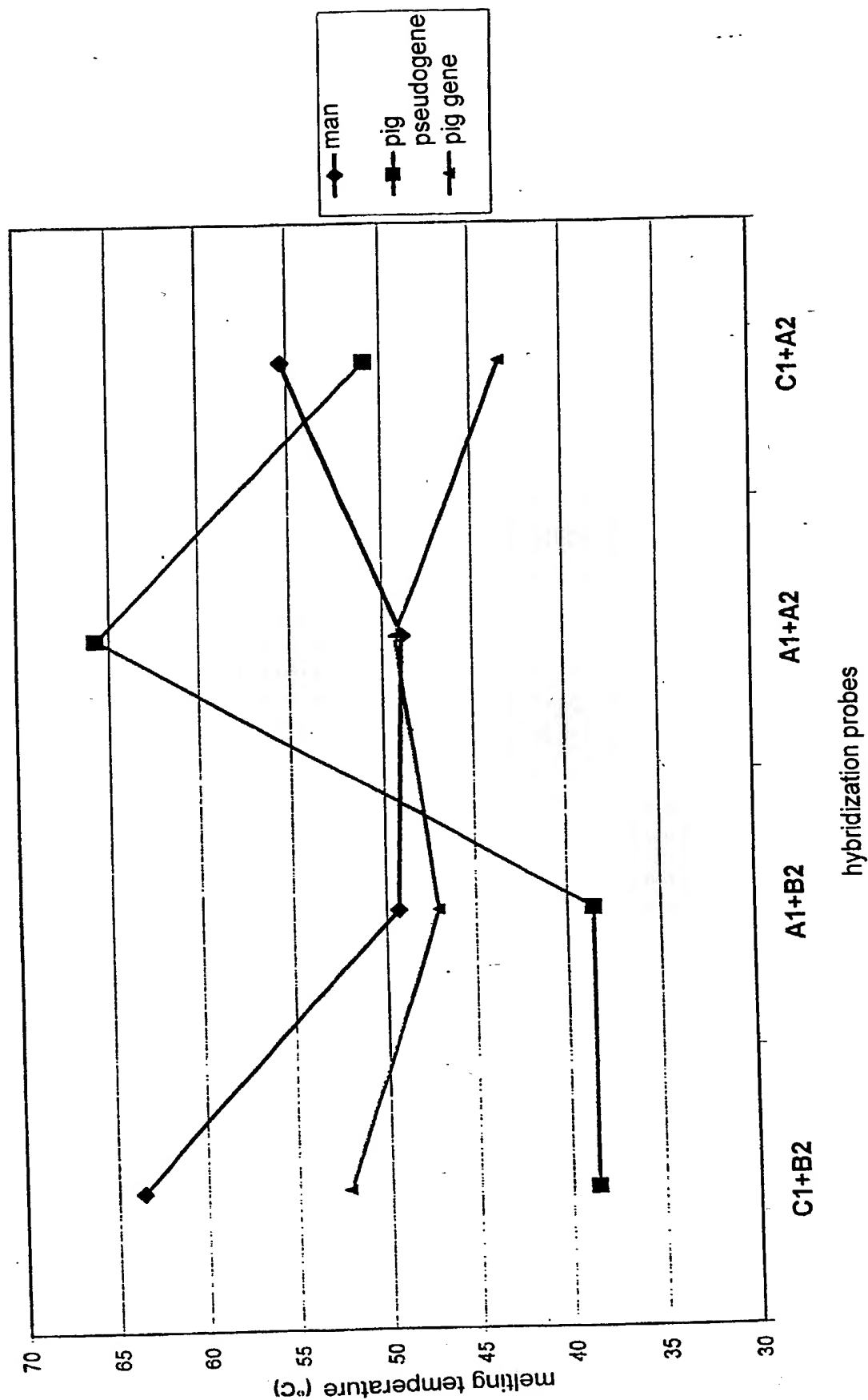


Figure 6: Melting point with probe A1 + A 2

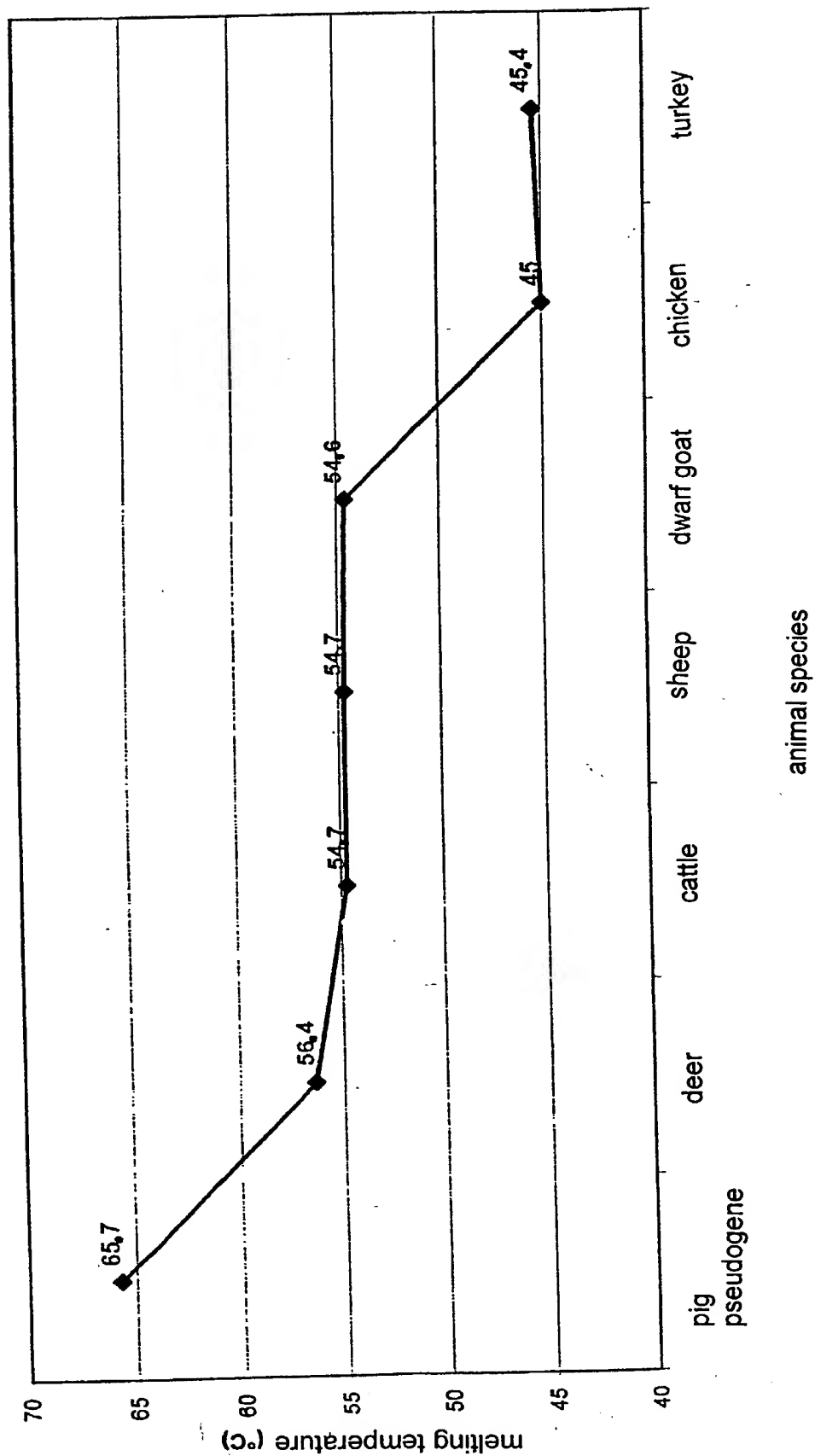


Figure 7: Melting point panel

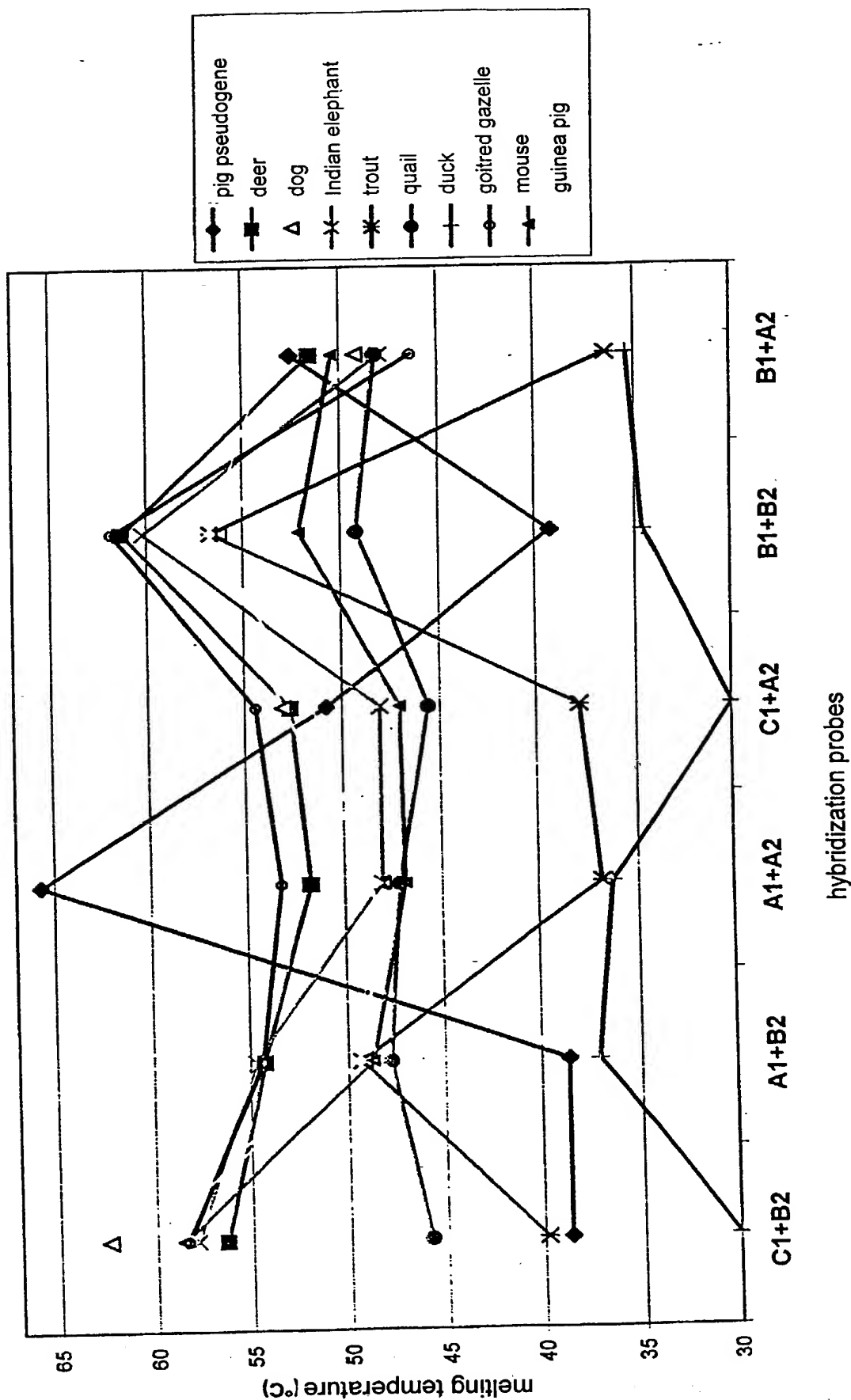
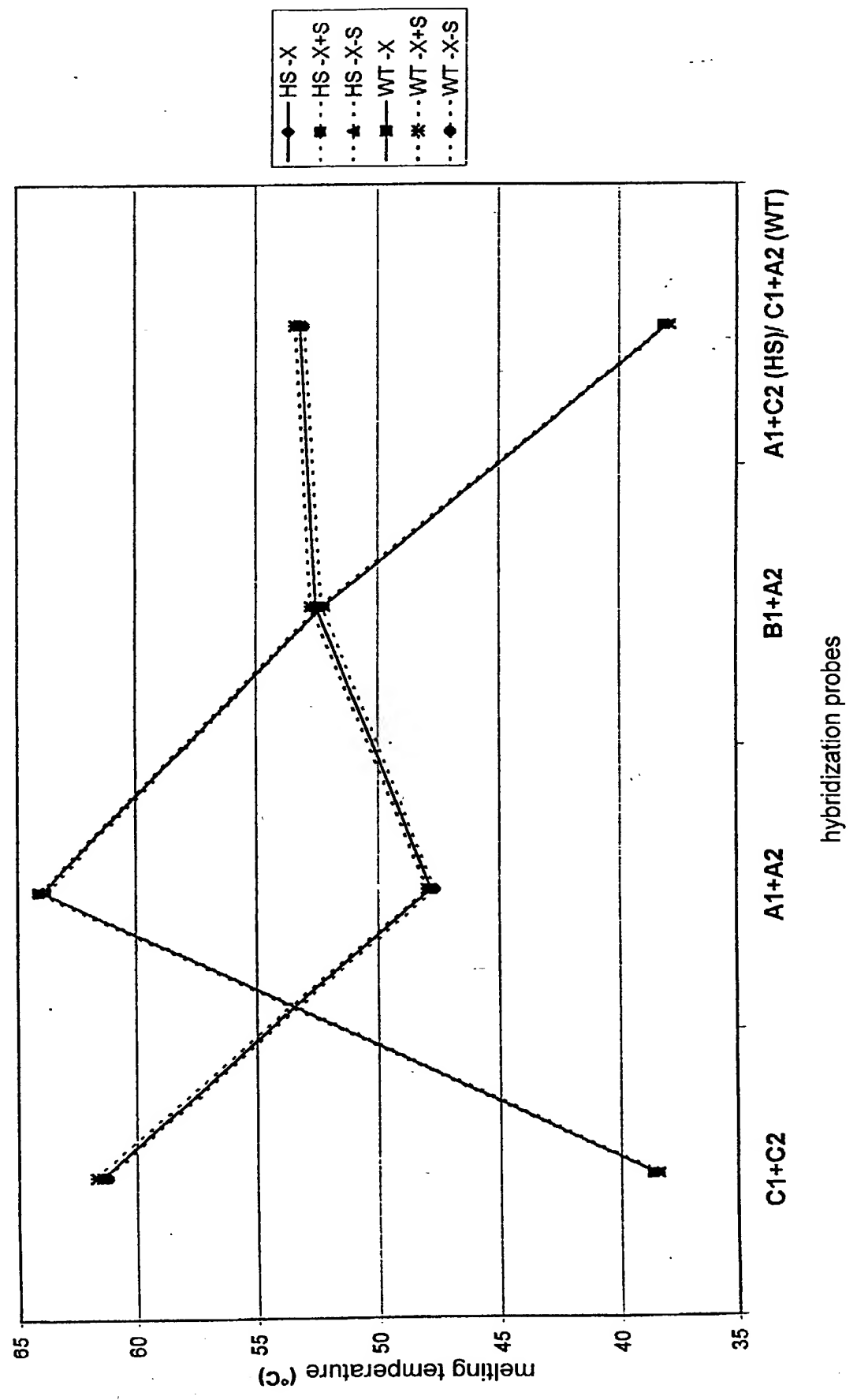


Figure 8: Standard deviations of select probes





TO ROBERT PATENT 19 FEB 2002  
 Attorney's Docket No. 012627-025

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Hans Konrad SCHACKERT <i>et al.</i>	)	Group Art Unit: Unassigned
	)	
Serial No.: 09/936,738	)	Examiner: Unassigned
Corresponding to PCT/EP00/02330	)	
	)	
International Filing Date: 3 March 2000	)	ATTENTION: BOX SEQUENCE
	)	
For: METHOD FOR IDENTIFYING	)	
ORGANISMS BY MEANS OF	)	
COMPARATIVE GENETIC	)	
ANALYSIS AND PRIMERS AND	)	
HYBRIDISATION PROBES FOR	)	
CARRYING OUT THIS METHOD	)	

**DECLARATION PURSUANT TO**  
**37 C.F.R. §§ 1.821-1.825**

Assistant Commissioner for Patents  
 Washington, D.C. 20231

Sir:


I, Deborah H. Yellin, declare as follows:

1. That the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same in compliance with § 1.821(f).
2. That the submission, filed in accordance with 37 C.F.R. § 1.821(g)[or (h)], herein does not include new matter [or go beyond the disclosure in the international application].

Serial No.: 09/936,738

I hereby declare that all statements made herein of my own knowledge are true and that all statements were made on information and belief and are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

February 22, 2002  
Date

  
Deborah H. Yellin  
Registration No. 30,427

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**  
(Includes Reference to Provisional and PCT International Applications)

Attorney's Docket No.

012627-025

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**METHOD FOR IDENTIFYING ORGANISMS BY MEANS OF COMPARATIVE GENETIC ANALYSIS AND  
PRIMERS AND HYBRIDIZATION PROBES FOR CARRYING OUT THIS METHOD**

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Number \_\_\_\_\_

on \_\_\_\_\_

and was amended

on \_\_\_\_\_ (if applicable).

☒ was filed as PCT international application

Number PCT/EP00/02330

on 16 March 2000

and was amended

on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:**

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Germany	199 11 656.3	16-03-99	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Germany	199 64 112.9	31-12-99	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
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I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

\_\_\_\_\_  
(Application Number)

\_\_\_\_\_  
(Filing Date)

\_\_\_\_\_  
(Application Number)

\_\_\_\_\_  
(Filing Date)



**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D)**  
(Includes Reference to Provisional and PCT International Applications)

Attorney's Docket No.

012627-025

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FULL NAME OF SIXTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			

09/936738

10 FEB 2002

## SEQUENCE LISTING

<110> Schackert, Hans Konrad  
Hahn, Matthias

<120> Method for Identifying Organisms by Means of Comparative Genetic  
Analysis and Primers and Hybridisation Probes for Carrying Out  
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<130> 012627-025

<140> US 09/936,738  
<141> 2001-09-17

<150> PCT/EP00/02330  
<151> 2000-03-16

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<151> 1999-12-31

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21

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 gctgggtggcg gggctctgca ggatggaaat ggctctggac ttggcggtag ctgatgcccc 180  
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 ggatggtggc agctactggg cctgcttctc ctacagcagc agangcctgg cagcggcggc 300  
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 agc 363

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 ctgcaacatg attgtcatct tcaacttagc attggtcaag atcttcacaa aagggtctga 180  
 taagtcttag ctgtgggtgg ttatgggtct caaaaggata ttgtgcaact gtggtaaaaa 240  
 gataacctca gaataagaaa aaaaaactct tgaattttta attancaagt aggnnnnttt 300  
 agaaatgttg catacaaaact taacagggtat ttaaaagaaa cactggattc cagagaaaaa 360  
 taatgtattg cttaactttc taattgttaa atagaaaata gtctcttgat aagtcttaaa 420  
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 gggaattttac gctatacgga ctggttagcat aggtcacata ttagaggtag agctaaactc 540  
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<210> 66  
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taga 64

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<211> 64  
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cttagac 67

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<212> DNA  
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<210> 72  
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<210> 75  
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<210> 86  
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<212> DNA  
<213> Domestic pig

<400> 86  
ccaaacatta ttgctatggg gtttcctgca gaaagacttg aaggcgtata caggaacaat 60  
attgatgatg 70

<210> 87  
<211> 71  
<212> DNA  
<213> Wild boar

<400> 87  
aaacattatt gctatggggg ttcctgcaga aagacttgaa ggcgtataca ggaacaatat 60  
tgatgatgta g 71

<210> 88  
<211> 63  
<212> DNA  
<213> Cattle

<400> 88  
cattattgct atgggctttc ctgcagaaag acttgaagggt gtatacagga acaatattga 60  
tga 63

<210> 89  
<211> 62  
<212> DNA  
<213> Sheep

<400> 89  
ttattgctat ggggtttcct gcagaaagac ttgaaggcgt gtacaggaac aatattgatg 60  
at 62

<210> 90  
<211> 58  
<212> DNA  
<213> Goat

<400> 90  
ttattgctat ggggtttcct gcagaaagac ttgaaggcgt gtacaggaac aatattga 58

<210> 91  
<211> 64  
<212> DNA  
<213> Red buffalo

<220>  
<221> misc\_feature  
<222> (1)...(64)  
<223> n = A,T,C or G

<400> 91  
cattattgct atgggggtttc ctgcagaaag acttgaaggc gtatnnagga acaatattga 60  
tgat 64

<210> 92  
<211> 68  
<212> DNA  
<213> Deer

```

<400> 92
tttatccaaa cattattgct atgggggtttc ctgcagaaag acttgaaggc gtatacagga 60
acaatatt 68

<210> 93
<211> 58
<212> DNA
<213> Roe deer

<220>
<221> misc_feature
<222> (1)...(58)
<223> n = A,T,C or G

<400> 93
ttattgctat ggggtttcct gcagaaagac ttgaaggcgt atannggaac aatattga 58

<210> 94
<211> 65
<212> DNA
<213> Goitred gazelle

<400> 94
ccaaacatta ttgctatggg gtttcctgca gaaagacttg aaggcgtata caggaacaat 60
attga 65

<210> 95
<211> 64
<212> DNA
<213> Horse

<400> 95
attattgcta tgggggtttcc tgcagaaaga cttgaaggcg tatacaggaa caatattgat 60
gatg 64

<210> 96
<211> 67
<212> DNA
<213> Dog

<220>
<221> misc_feature
<222> (1)...(67)
<223> n = A,T,C or G

<400> 96
ttccaaacat tattgctatn ggggtttcctg cagaaagact tgaaggcgta tacnggaaca 60
atattga 67

<210> 97
<211> 65
<212> DNA
<213> Sun bear

<220>
<221> misc_feature
<222> (1)...(65)

```



<223> n = A,T,C or G

<400> 97

tccaaacatt attgctatng ggtttcctgc agaaagactt gaaggcgtat acaggaacaa 60  
tattg 65

<210> 98

<211> 62

<212> DNA

<213> Rabbit

<400> 98

gctatgggat ttcctgcaga aagacttgaa ggcgtataca ggaacaatat tgatgatgta 60  
gt 62

<210> 99

<211> 59

<212> DNA

<213> Hare

<400> 99

acattattgc tatgggattt cctgcagaaa gacttgaagg cgtatacagg aacaatatt 59

<210> 100

<211> 48

<212> DNA

<213> Antelope

<400> 100

ttgctatggg gtttcctgca gaaagacttg aaggcgtata caggaaca 48

<210> 101

<211> 77

<212> DNA

<213> Turkey

<400> 101

tttatccaaa cattattgct atgggttttc ctgcggagag gcttgaagga gtataccgga 60  
acaatattga tgatgta 77

<210> 102

<211> 73

<212> DNA

<213> Chicken

<400> 102

atttatccaa acattattgc tatgggtttt cctgcggaga ggcttgaagg agtataccgg 60  
acaatattg atg 73

<210> 103

<211> 61

<212> DNA

<213> Duck

<400> 103

ttattgctat gggttttcct gcagagaggc ttgaaggagt gtaccggaac aatattgatg 60  
a 61

<210> 104  
<211> 62  
<212> DNA  
<213> Quail

<400> 104  
cattattgct atggggttttc ctgcggagag gcttgaagga gtataccgga acaatattga 60  
tg 62

<210> 105  
<211> 73  
<212> DNA  
<213> Goose

<400> 105  
tttatccaaa cattattgct atggggttttc ctgcagagag gcttgaagga gtgtaccgga 60  
acaatattga tga 73

<210> 106  
<211> 66  
<212> DNA  
<213> Ostrich

<400> 106  
ccaaacatta ttgctatggg ttttccggcg gagaggcttg aaggagtgtg ccggaacaat 60  
attgat 66

<210> 107  
<211> 59  
<212> DNA  
<213> Pigeon

<400> 107  
cattattgct atggggttttc ctgcggagag gcttgaagga gtataccgga acaatattg 59

<210> 108  
<211> 60  
<212> DNA  
<213> Varan

<400> 108  
cattattgct atggggttttc ctgcggagag gcttgaagga gtataccgga acaatattga 60

<210> 109  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zoo43sUV

<400> 109  
tgtgctgaga gacattatga c 21

<210> 110  
<211> 20

<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zoo44aRV

<400> 110  
ttgtctctgg tccttacttc 20

<210> 111  
<211> 654  
<212> DNA  
<213> Man

<400> 111  
ttatgacacc gccaaattta attgcagagt atgaatgtac tgtactatgt tgtataactt 60  
aaacccgata gactgtatct tactgtcata acaataatga gtcattccaga ttatcgagtg 120  
agatacatat ttaagaatta tctttaaaaa tttcaaaaat ttttaatttta ctgttggtgt 180  
ttaggaaaaa gtattgcata aagctattaa tattgtcagg aagactaaag tgcagcatag 240  
actaagaatt aggaaaattc ctagactaaa aatagtataa ggagagggtt tacctactat 300  
ttgaggcagt tgggtctaata gtaagcaatc acagggagaa agcagaacta cttaactctt 360  
ctgtgttgag gaatgacata aaaggtagga aaggatataa caaatgttga taagaggagt 420  
ctgatggatg agaggaggga actgctttaa atgagtttct acttcagaca taagttaatt 480  
ctcagagccc acaaaaactt tcaacttttat ttgtgaaata caactcagtt ctcatggctt 540  
aacactttta accatgagaa aactgaagag ttgagagctt ggcagatgct gctgtgatag 600  
tcaaaagaaa gtgggtgcat gagctactat tgatgtattt gccatggtcc ctcc 654

<210> 112  
<211> 582  
<212> DNA  
<213> Dog

<400> 112  
atgtaataaa tatgcacaaa tcattacacc agttcgtccc tttccagctt tacagtgaat 60  
tgctgcaaca tgattgtcat cttcacttag ccattgggtca agatcttcac aaaagggttt 120  
gataagttct agctgtggtg gattatggtc ttcaaaagga tactgtgcaa ctgtggtaaa 180  
aagataacct cagaattaga aaaaagtctt tccgtgaactg tttattaaaa gtaggttaac 240  
tttagaaaca ttgcatgtaa gcttaacaga tgtttaaaag aaaaacggaa ctccagagaa 300  
aaataatttg ctgtctgata attttccaat ttttgaatag aaaatagtct ctcattaatt 360  
cttaaaccta ccaactadgag agagaggcta agcattattt tccccactt taatgaaaga 420  
ggaaactttg caatggagag ggagcacacg tcaacatata agagggaaga ggcaaactca 480  
aatgaaatg gcacacaggt ttctgtcag ggctctcaat gcattttctg acaaaaggag 540  
tcataatatt tataatacta cgtcatccaa aatatatatt cc 582

<210> 113  
<211> 376  
<212> DNA  
<213> Cattle

<220>  
<221> misc\_feature  
<222> (1)...(376)  
<223> n = A,T,C or G

<400> 113  
taggtacaca tattgtgtta gataacttga agccaacagt ctaaatttta ctgtcatacc 60  
aataatgaat aatctcaagt attaagtgat atatttatct taaagatggt ctgagaaaat 120

```

ttgaaattaa ttttgcgtgt gtgttttttg aaataagtat catgtaaatg aggaagacta 180
aattgaatta actgaaaact aggagaaatt tatagactaa cagaataaat agagggttat 240
atctgtgatt tgaggcattt ggcatgatag taagagatta caggggagaa aggagaatgg 300
cttaattctg taatggaaca tgacctgtac agtgggaaaa ggggtataat gaantatgga 360
tnaaaaggag cctgaa 376

```

<210> 114  
 <211> 673  
 <212> DNA  
 <213> Mouse

```

<400> 114
ttatgacacc gccaaattta actgcagagg tatgtataaa cataaccaca gcatactgta 60
taactaaaga ccaatagact tgtctttttac tgcctgggtga taattatcaa gattagttag 120
ataaaaatct taagaatggc ctttgacaat taaaaaaagt gtattttaatg ttagagttgt 180
tctttaagac ctatctattg tcaggaaaaac taaatcacag aatacttga gaggtcccaa 240
gactaaaacta ggattggagg tgcttattga cgggtgtggga cagctagcgc tgctggaaac 300
aatcacaga agagagcaga accatttttaa cttttctaca tcgaagaatg gcataaaagt 360
agggaaagat gtatcatcgg tctgtctgtc tgtctgtctg cctgtctgtc ttctcagaat 420
catgaagcac taaggagtaa gtaagaacag tttctggggg accgacagac ctaggctact 480
gtcattagg aaacatgcca tggttgaagg tcacttagct ttaaattgtac attttaacag 540
actcttgaat gttcttgtgt gccactgggg gaaatgaggt cgggagcaca gtttagacaga 600
tggtttaagta aaagctggcc tgcagcctct tgggtgaatgt agtttgccat tgtttaccac 660
agagctttcc tgt 673

```

<210> 115  
 <211> 411  
 <212> DNA  
 <213> Horse

```

<400> 115
aatgtacagt attttgttat ataactgaaa accagtagac taagtcttac tgtcacagca 60
gtaatgaata ctcttgatta ttaagtgaga taaatattta tcttaaaaag ataactcttag 120
aaaatttgaa aaataaattt aactttgctg ttgtatttta gaaaacaagt atcatataaa 180
ccaactggta gtattaggaa gactaaattg aagaatagac taagaattag gatgtaatat 240
taagagattg catggagaaa gcagaacgac ttaactctgg caaggagcgt gacctaaaag 300
gtggaaaagg gtataacaga tgtggatata aggagcctga acagatgaga gcagggaact 360
gcttcaaatg agttcttttc caagtatagt aaattgtttc tcagagccca c 411

```

<210> 116  
 <211> 566  
 <212> DNA  
 <213> Sheep

<220>  
 <221> misc\_feature  
 <222> (1)...(566)  
 <223> n = A,T,C or G

```

<400> 116
aaaaatttgc nnnngatgta acaaatatgc acaaatcatt acaccagttc gtcccttttc 60
agcttttacag tgaattgctg caacatgatt gtcattctca cttagccatt ggtcaagatc 120
ttcacaaaag ggtttgataa gttctaactg ttgggtggatt atgggtctca aagggatact 180
gtgcaactgt gataaaaaaga taaccgcaga tatatgaaaa taatctcact tgaattgctt 240
attacaagta ggctaacttt agaaatgttg catacaaaata gtttaaaaat gtctgaacta 300
tagaggaaaa gaatttattg tctgataatt ttctaatttt cgaacagaaa ataactctct 360
attaactcaa atttatccat tcgacaggta agacaagtat tatttctctca ctctatgatg 420

```

gaggcaatgg aggagcaaca tatcagaggt cacaacataa cggaggaaga ggcaaactca 480  
gaatgaaacg tcgcacgagc ctcttagcag ggctctcaat acgttcctag caaaaggagc 540  
tggtaacatc tataatatcg cattat 566

<210> 117  
<211> 497  
<212> DNA  
<213> Turkey

<400> 117  
aagctgcatt ttgccaggtg taaggaactg acagagacaa ccaagaccaa agcattttcag 60  
gctgaattcc cctckttcct cccacctcct ctgaacaaat ggaggttctg acagagtggg 120  
gagattaatt cagaatatgt gtgcacagta cacctggcag accccacaaa gcttggctca 180  
aagaacaaag atgaaacaaa ggcatgaata gagcagtaga aggattttaca aaaggacaaa 240  
agatgggcag ccattttaaag gtgacagtaa ttctttaagt aaatgtcaaa actcttcaaa 300  
gaagcaaggg ggataaatatt catgaatact taaggctgaa acgtgaacat gttgatttgc 360  
catttggaag gtlatgtttc cttcttatct cctctctgat agcttcaata atgggcacta 420  
aaattcgttc ctgaaaaaat gcaaagaaat cactcagtggt ctgaggacgt gttgatttca 480  
catgtattga aatcagt 497

<210> 118  
<211> 365  
<212> DNA  
<213> Trout

<220>  
<221> misc\_feature  
<222> (1)...(365)  
<223> n = A,T,C or G

<400> 118  
cattatgacn nnnnnnnnatt caattgcaga ggattagata ttacatcaga gtgaaaccat 60  
tatcactgtc ttccaggcag tcagtgaatg aatcaatctt tcactaaaaa cccacgtgtg 120  
acgtactaact actgagcccg gtctctgtct gtctctctcc agttgcacaa tatccggttg 180  
aggatcacaa tccgccccag ctggagctga tcaaacctgt ctgcgaagat cttggccttt 240  
ggttaagtga agacgacaat catgtggcgg cgattcactk taaarctgga aaggacgtac 300  
gggtgtcatg atctgtgctt acctgttaca cgggggcaag ttcttcaaag cacaagaagc 360  
tctcg 365

<210> 119  
<211> 656  
<212> DNA  
<213> Roe deer

<400> 119  
gtataggtac acttactatg ttagataact tgaggccaac agtctaaatt ttactatcat 60  
accagtaatg aataatctca agtattaagt gatacagtea tcttaaagat gatcttagaa 120  
aatttgaaat taattttgct gttgtgtttt tggaacaaag tgtcatgtaa atgagggaga 180  
ctaaactgaa ttaactgaaa actaggagaa atttatagac tgacagaata aagaaagggt 240  
tatatctgtg atttgaggca ttggcggtaa taglaagaga ttacaggag aaaggagaat 300  
gatttaattc tataatggaa catgacctgc acagtggaaa aaggggtataa tgaaatataa 360  
awaaaaggag cctgatagat gagagcaaga actgctttta gtgaattttt ctccagggtat 420  
agtatatatt atctcagagt ccacaaatac ttctatttgt ttttgtggaa ctcttagaac 480  
gacgagagac caggaacatt gagaagctaa tatatttgcc attgttccct cctaaatatt 540  
tagcacaggc ttccaacag ttggttttaag aattcagaag tgctaataac tgagagcaag 600  
ggtagattta ttactaagaa tgtttcattt ttgggtggatt ttgctatttc tggta 656

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<210> 120  
<211> 405  
<212> DNA  
<213> Deer

<220>  
<221> misc\_feature  
<222> (1)...(405)  
<223> n = A,T,C or G

<400> 120  
gtataggtac acttttnnaag ccaacagtct aaatttttact gtcataccaa taatgaataa 60  
tctcaagtat taagtgatat atttatctta aagatgatct tagaaaaattt gaaactaatt 120  
ttgctgttgt gtttttggaa acaagtgtca tgtaaatgag ggagaccata actgaattaa 180  
ctgaaaactg ggaaaaattt atagactaac agaataaaga aaggggtata tctgtgggtt 240  
gagggcgtttg acgtaatagt aagagattac agggagaaaag gagaatgact taattctata 300  
atggaacacg acctgcacag tggaaaaagg gtataatkaa atgtagataa aggagcctga 360  
tagttgagag caagaactgc ttttaagtgc tttttctcca ggtgt 405

<210> 121  
<211> 522  
<212> DNA  
<213> Chimpanzee

<220>  
<221> misc\_feature  
<222> (1)...(522)  
<223> n = A,T,C or G

<400> 121  
cattatgacn nnnnnnnnnn nnattgcaga ggtaggtatg aatgtactgt actatgttgt 60  
ataacttaaa cccgatagac tgtatcttac tgtcataaca ataatgagtc atctagatta 120  
tcgagtgaga tacatatatta tcttaagaat tatcttttaa aatttcaaaa attttaattt 180  
tactcttgtg ttttaggaaa aaagtattgc ataaagctat taatattgtc aggaagacta 240  
aagtgcagca tagactaaga atgaggaaaa ttcctagact nnaatagtag aaggagaggg 300  
tttacctact atttgaggca gttgggtctaa tagtaagcaa tcacagggag aaagcagaa 360  
tacttaactc ttctgtgttg aggaatgaca taaaaggtag gaaggatata acaaatgttg 420  
ataagaggag tctgatggat gagaggaggg aactgcttta aatgagttct acttcagaca 480  
tadgttaatt ctcagagccc acaaaacttt cactttttatt tg 522

<210> 122  
<211> 666  
<212> DNA  
<213> Gorilla

<220>  
<221> misc\_feature  
<222> (1)...(666)  
<223> n = A,T,C or G

<400> 122  
cattatgacn nnnnnnnnatt taattgcaga ggtaggtatg aatgtdctgt actatgttgt 60  
ataacttaaa cccgatagac tgtatcttac tgtcataaca ataatgagtc atctagatta 120  
tcgagtgaga tacatatatta tcttaagaat tatcttttaa aatttcaaaa attttaattt 180  
tactcttgtg ttttaggaaa aaagtattgc ataaagctat taatattgtc aggaagacta 240  
aagtgcagca tagactaaga atgaggaaaa ttcctagact nnaatagtag taaggagagg 300  
gtttacctac tatttgagga agttgggtcta atagtaagca atcacaggga gaaagcagaa 360

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```
ctactttaact cttctgtgtt gaggaatgac ataaaaggta ggraaggata taacaaatgt 420
tgataagagg rgtctgatgg atgagaggag ggaactgctt taaatgagtt ctacttcaga 480
cataagttaa ttctcagagc ccacaaaaaac ttctcactttt atttgtgaaa tgcaactcag 540
ttctcatggc ttaacactttt aamccatgag agactgaaga gttgagaagc ttggcagatg 600
ctgctgtgat agtcaaaaaa aaagtgggtg ccatgagcta ctattgatgt atttgccatt 660
gatccc 666
```

```
<210> 123
<211> 741
<212> DNA
<213> Orang-utan
```

```
<220>
<221> misc_feature
<222> (1)...(741)
<223> n = A,T,C or G
```

```
<400> 123
cattatgacn nnnnnaaatt taattgcaga ggtagggtacg aatgtactgt gctatgttgt 60
ataacttaaa cacaatagac tgtatcttac tgtcataaca ataatgactc atctagatta 120
ttgagtgaga tacatatatta tcttaagawt tatcttaaaa aatttcagaa aatttaattt 180
tactgttgtg ttttaggaaa aacgtattgc ataaagctat taatattgtc aggaaaagtg 240
cagagtagac taagaattag gaaaattcct agactaaaan nnnataagga gagggtttac 300
ctactgtttg aggcagttgg tctaatagta agcgattata gggagaaaagc agaactactt 360
aactcttctg tgttgaggaa tgacatgaaa ggtaggaaaag gatataacaa atgttgataa 420
gaggagcctg atggatgaga ggaggggaact gctttaaatg agttctactt cagacataag 480
ttaattctca gagccacaaa aaactttcac ttctatttgt gaaatacaac tcagttctca 540
cggcttaaca ctttaaacca tgagagaact gaagagttga gaagccttggc agatgcttct 600
gtgatagtca aaaagaaaagt ggggtccatg agctactatt gatgtatttg ccattgatcc 660
cycctgaaaa tctagaatgg actttcagac aaatggtttg aaaatcctaa atcactaatg 720
attgggattt agtatagatt c 741
```

```
<210> 124
<211> 608
<212> DNA
<213> Orang-utan
```

```
<220>
<221> misc_feature
<222> (1)...(608)
<223> n = A,T,C or G
```

```
<400> 124
cattatgacn nnnncaaatt taattgcaga ggtagggtacg aatgtactgt gctatgttgt 60
ataacttaaa cacaatagac tgtatcttac tgtcataaca ataatgactc atctagatta 120
ttgagtgaga tacatatatta tcttaagaat tatcttaaaa datttcagaa aatttaattt 180
tactgttgtg ttttaggaaa aacgtattgc ataaagctat taatattgtc aggaaaagtg 240
cagagtagac taagaattag gaaaattcct agactaaaan nnnataagga gagggtttac 300
ctactgtttg aggcagttgg tctaatagta agcgattata gggagaaaagc agaactactt 360
aactcttctg tgttgaggaa tgacatgaaa ggtaggaaaag gatataacaa atgctgataa 420
gaggagcctg atggatgaga ggaggggaact gctttaaatg agttctactt cagacataag 480
ttaattctca gagccacaaa aactttcact ttctatttgt aaatacaact cagttctcac 540
ggcttaacac ttttaacccat ggagagacct gaagaglttg agaagccttg cagatgcttc 600
tgtgatag 608
```

```
<210> 125
<211> 402
```

<212> DNA

<213> Banting cattle

<400> 125

```
gagagacatt atgacaccgc caaattttaat tgcagaggta agtataggta cacatattat 60
gttagataac ttgaagccaa cagtctaaat tttactgtca taccaataat gaataatctc 120
aagtattaaag tgatatatatt atcttaaaga tggctcgaga aaatttgaaa ttaattttgc 180
tggtgtgttt ttggaaataa gtatcatgta aatgaggaag actaaattga attaaactgaa 240
aactaggaga aatttataga ctaacagaat aaatagaggg ttatatctgt gatttgaggc 300
atgtggcatg atagtaagag attacaggga gaaaggagaa tggcttaatt ctgtaatgga 360
acatgacctg tacagtggaa aaggggtataa tgaaatatgg at 402
```

<210> 126

<211> 479

<212> DNA

<213> Indian elephant

<220>

<221> misc\_feature

<222> (1)...(479)

<223> n = A,T,C or G

<400> 126

```
gacattatga cnnnnnnnnnn nnnnnntgca gaggtaggta taaatgtttt atagtatggt 60
gtataactta aaaccaaag tctaaatatt actgccatag caatagtga tttcttagat 120
tattaagtaa gataaatatt tatcttaagg atggctctta aaatttgagg gaaataaatt 180
taattttaat attatgtttt agaacaagta tcccataacc ctatgagtaa tgcctgaag 240
accaaaataa agaattaggc aagaattagg agaaattcct aggataagaa taaaataagg 300
aaggggggca tgcctagtgt ttgaggcagt tgggtgtaata ctaagagatt atatggagaa 360
agcaggacta ctcaattctt ctctatcaaa gagaataacc taaaggggtg aaaagagtat 420
aacaaatgtg gataagagga gcttgagAAC gagagtgggg agatgcttta aatgagctc 479
```

<210> 127

<211> 284

<212> DNA

<213> Fishing cat

<400> 127

```
gagagacatt atgacaccgc caaattttaac tgcagaggta ggtattaaht gcagagtaat 60
gtattatggt atataactyc aaaccagtag actaaactct actgtcatag cagtgatgaa 120
taatctcatt attaatgtg ataaatattt atcttcaaga tggctcttaa aaatttgcaa 180
aacaaattta attttgctgt tgtgttttg gaagcaagta tcctataaac ctgccggtac 240
taactagtag gaagactaat cccagagtag actaagaatt tgga 284
```

<210> 128

<211> 290

<212> DNA

<213> Sun bear

<220>

<221> misc\_feature

<222> (1)...(290)

<223> n = A,T,C or G

<400> 128

```
gagagacatt atgacnnnnn nnnnnnnaac tgcagaggta ggtaaaaact gccaaagtaat 60
gtatttatgt tgtataactt aaaaccagta gaccaaactt tactatcata gcagtaatga 120
```



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ataatctcaa ttaattaagt ggaagtaa atttatctt aaagatggc ttagacactt 180  
 tggaaaacta atttaatat gctgttgtgt tttaggaagc agttatcata taaacctgcc 240  
 agtactagta cgaataactaa aacgcagagt agactctaaa attgaggaaa 290

<210> 129  
 <211> 272  
 <212> DNA  
 <213> Dwarf goat

<400> 129  
 gagagacatt atgacaccgc caaattta atgacagagta agtacagga cacatattat 60  
 gttaggtaac ttgaagccaa cagtctaa tttactgtca taccaataat gaataatcac 120  
 aagtattaa taatatatt atgttaaaga tggcctgaga aaatgtgaaa ttaactttgc 180  
 tgttgtgttt ttggaaataa gtatcatgta aatgaggatg actaaattga attaactgaa 240  
 aactaggaga agtttataga ctaacagaat ag 272

<210> 130  
 <211> 327  
 <212> DNA  
 <213> Guinea pig

<220>  
 <221> misc\_feature  
 <222> (1)...(327)  
 <223> n = A,T,C or G

<400> 130  
 gagagacatt atgacnnnnn nnnattta atgacagagta tgtataaata taccatggc 60  
 tggggatga ttgaaaacca ataggctgtg tttattatc agcaataatg gatcatttaa 120  
 attattagaa aagataaata tttttcttta attatagtct gagataattt gaaaatacta 180  
 attttttggt tgagctttag aaatcatgtg tcaggtaa atctgtcaatgt tgtccggaaa 240  
 actcgagtac atagtagact taagaattag gataaattac taaactgata atggaataaa 300  
 gaggatattt acctgctgct tgaaaca 327

<210> 131  
 <211> 21  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Zoo43sUV

<400> 131  
 tgtgctgaga gacattatga c 21

<210> 132  
 <211> 19  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Zoo44aRV

<400> 132  
 ttgtctctgg tccttactt 19

<210> 133

<211> 281  
<212> DNA  
<213> Man

<400> 133  
ttgtctctgg tccttacttc cccatagaaa tctagggcct cttgtgcctt taaaaatttg 60  
ccccgatgta ataaatatgc ataaatcatt ataccagttc gtccctttcc agcttttacag 120  
tgaattgctg caacatgatt gtcattcttca cttagccatt ggtcaagatc ttcacaaaag 180  
ggtttgataa gttctagctg tgggtgggta tggctttcaa aaggatattg cgcaactctg 240  
taattagatt tggcgggtgc ataattgtctc tcagcacaaac t 281

<210> 134  
<211> 271  
<212> DNA  
<213> Chimpanzee

<400> 134  
ggctcttact tccccataga aatgttagggc ctcttgtgcc tttaaaaatt tgccccgatg 60  
taataaatat gcataaatca ttataccagt tcgtcccttt ccagctttac agtgaattgc 120  
tgcaacatga ttgtcatctt cacttagcca tcgggtcaaga tcttcacaaa aggggtttgat 180  
aagttctagc tgtgggtggg tatgggtcttc aaaaggatat tgcgcaactc tgtaattaga 240  
tttggcggtg tcataatgtc tctcagcaca a 271

<210> 135  
<211> 271  
<212> DNA  
<213> Oran-utan

<220>  
<221> misc\_feature  
<222> (1)...(271)  
<223> n = A,T,C or G

<400> 135  
tggtccttac ttccccatag aaatctaggg cctcttgtgc ctttaaaaaat ttgccccgat 60  
gtaataaata tgcacaaatc attacaccag ttcgctccctt tccagcttta cagtgaattg 120  
ctgcaacatg attgtcatct tcacttagcc attgggtcaag atcttcacaa aagggtttga 180  
taagttctag ctgtgggtgg ttatggctct caaaaggata ttgtgcaact nnnnnnnnnn 240  
nnnnnnnnnn gtcataatgt ctctcagcac a 271

<210> 136  
<211> 268  
<212> DNA  
<213> Gorilla

<400> 136  
ctggctcctta ctccccaga gaaatctagg gcctcttgtg cctttaaaaa tttgccccga 60  
tgtaataaat atgcataaat cattatacca gtctgtccct tccagctttt acagtgaatt 120  
gctgcaacat gattgtcatc ttcacttagc cattgggtcaa gatcttcaca aaagggtttg 180  
ataagttcta gctgtgggtg gttatggctt tcaaaaggat attgtgcaac tctgcaatta 240  
aatttggcgg tgtcataatg tctctcag 268

<210> 137  
<211> 306  
<212> DNA  
<213> Domestic pig

<400> 137  
tctctggtcc ttacttcccc atagaaatct tgtgccttta aaaatttgcc cggatgaaac 60  
aaatatgcac aaatcattac accagttcat ccttttccag gtttacagtg aattgctgca 120  
acatgattgt catcttcact tagccattgg tcaagatctt cacaaaaagg tttgataaat 180  
tctagctgtg gtggattatg atcttcaaaa ggatactgtg caactctgca gttaaatgtg 240  
gcggtgtcat aatgtctctc agcacaaactc tgcaattaaa tttggcggtg tcataatgtc 300  
tctcag 306

<210> 138  
<211> 258  
<212> DNA  
<213> Wild boar

<400> 138  
tctctggtcc ttacttcccc atagaaatct tgtgccttta aaaatttgcc cggatgaaac 60  
aaatatgcac aaatcattac accagttcat ccttttccag gtttacagtg aattgctgca 120  
acatgattgt catcttcact tagccattgg tcaagatctt cacaaaaagg tttgataaat 180  
tctagctgtg gtggattatg atcttcaaaa ggatactgtg caactctgca gttaaatgtg 240  
gcggtgtcat aatgtctc 258

<210> 139  
<211> 18  
<212> DNA  
<213> SPL5 senseArtificial Sequence

<220>  
<223> SPL5 sense

<400> 139  
aaatttaatt gcagaggt 18

<210> 140  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zoo44aRV antisense

<400> 140  
ttgtctctgg tccttacttc 20

<210> 141  
<211> 712  
<212> DNA  
<213> Man

<400> 141  
ttgtctctgg tccttacttc cccatagaaa tctagggcct cttgtgcctt taaaaatttg 60  
ccccgatgta ataaatatgc acatatcatt acaccagttc gtccctttcc agcttttacag 120  
tgaattgctg caacatgatt gtcattctca cttagccatt ggtcaagatc ttcacaaaaag 180  
ggtttgataa gttctagctg tgggtgggta tgggtcttcaa aaggatattg tgcaactgtg 240  
gtaaaaagat aacctcagaa taagaaaaaa aaactcttga atttttaatt aacaagtagg 300  
taactttaga aatgttgcac acaaacttaa caggatatta aaagaaacac tggattccag 360  
agaaaaataa tgtattgctt aactttctaa ttgttaaata gaaaatagtc tcttgataag 420  
tcttaaatat aatcattaag gaagccaggc attattttcc cccattttat tcaggaggat 480  
atattctggg aatttacgct atacggactg gtagcatagg tcacatatta gaggtagagc 540

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taaaccctaaa atgaactgtc acatggacat ttcgtcagga ctctcaatgc aaaaggaata 600  
atactattta tagtatttat ttcacatca caaaacatat tccaaagaca gaatagtta 660  
ctaataggta aactatgcaa agaactacat attacatttc ataaaataaa aa 712

<210> 142  
<211> 593  
<212> DNA  
<213> Chimpanzee

<220>  
<221> misc\_feature  
<222> (1)...(593)  
<223> n = A,T,C or G

<400> 142  
tggtccttac ttcccatag aaatctaggg cctcttgtgc ctttaaaaat ttgccccgat 60  
gtaataaata tgcacaaatc attacaccag ttcgtccctt tccagcttta cagtgaattg 120  
ctgcaacatg attgtcatct tcacttagcc attggtcaag atcttcacaa aagggcttga 180  
taagttctag ctgtggtggg ttatggtctt caaaaggata ttgtgcaact gtggtaaaaa 240  
gataacctca gaataagaaa aaaaaactct tgaattttta attancaagt aggnnnnttt 300  
agaatgttgc atacaaactt aacaggtatt taaaagaaac actggattcc agagaaaaat 360  
aatgtattgc ttaactttct aattgttaaa tagaaaatag tctcttgata agtcttaaat 420  
ataatcatta aggaagccag gtattattct cccccatttt attcaggagg atatatctg 480  
ggaattttacg ctatacggac tggtagcata gggtcacatat tagaggtaga gctaaactca 540  
aaatgaactg tcacatggac atttcatcag gactctcaat gcaaaaggaa taa 593

<210> 143  
<211> 589  
<212> DNA  
<213> Chimpanzee

<220>  
<221> misc\_feature  
<222> (1)...(589)  
<223> n = A,T,C or G

<400> 143  
ccttacttcc ccatagaaat ctagggcctc ttgtgccttt aaaaatttgc cccgatgtaa 60  
taaataatgca caaatcatta caccagttcg tccctttcca gctttacagt gaattgctgc 120  
aacatgattg tcatcttcac ttagccattg gtcaagatct tcacaaaagg gtttgataag 180  
ttctagctgt ggtgggttat ggtcttcaaa aggatattgt gcaactgtgg taaaaagata 240  
acctcagaat aagaaaaaaa aactcttgaa tttttaatta acaagtaggn nntttagaaa 300  
tgttgcatac aaacttaaca ggtattttaa agaaacactg gattccagag aaaaaaatg 360  
tattgcttaa ctttctaatt gttaaataga aaatagtctc ttgataagtc ttaaatataa 420  
tcattaaggg agccaggtat tattctcccc cattttattc aggaggatat attctgggaa 480  
tttacgctat acggactggg agcatagggt acatattaga ggtagagcta aactcaaaat 540  
gaactgtcac atggacattt catcaggact ctcattgcaaa aggaataat 589

<210> 144  
<211> 593  
<212> DNA  
<213> Orang-utan

<400> 144  
acttccccat agaaatctag ggcctcttgt gccttttaaaa atttgccccg atgtaataaa 60  
tatgcacaaa tcattacacc agttcgtccc ttccagctt tacagtgaat tgctgcaaca 120  
tgattgtcat cttcacttag ccattgggtca agatcttcac aaaagggttt gataagttct 180

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```

agctgtggtg gggttatggtc ttcaaaaagga tattgtgcaa ctgtgggtaaa aagataacct 240
cagaataaga aaaaaaaact cctgaatttt tcattaacaa gtaggtaact ttagaaatgt 300
tgcatacaaa cttaacaggt atttaaaaga aacactggat tccaaagaaa aataatgtat 360
tgcttaactt tctaattggt aaatagaaaa tagtctcttg ataagtctta aatataatca 420
ttaaggaagc caggtattat tttcccccac tttattcagg aggatatatt ctgggggattt 480
acactatacg gactggtagc ataggtcaca tattagaggt agagctaaac ccaaaatgaa 540
atgtcacatg gacatttcgt caggactgtc aatgcaaaag gaataatact att 593

```

<210> 145  
 <211> 724  
 <212> DNA  
 <213> Orang-utan

```

<400> 145
tccttacttc cccatagaaa tctagggcct cttgtgcctt taaaaatttg ccccgatgta 60
ataaatatgc acaaatcatt acaccagttc gtccctttcc agctttacag tgaattgctg 120
caacatgatt gtcactttca cttagccatt ggtcaagatc ttcacaaaag gggtttgataa 180
gttcttagctg tgggtgggta tgggtcttcaa aaggatattg tgcaactgtg gtaaaaaagat 240
aacctcagaa taagaaaaaa aaactcctga atttttcatt aacaagtagg taactttaga 300
aatgttgcat acaaaactta caggtattta aaagaaacac tggattccaa agaaaaataa 360
tgtattgctt aacttttctaa ttgttaaata gaaaatagtc tcttgataag tcttaaatat 420
aatcattaag gaagccaggt attattttcc cccattttat tcaggaggat atattctggg 480
aatttacact atacggactg gtagcatagg tcacatatta gaggtagagc taaacccaaa 540
atgaaatgtc acaggacatt tcgtcaggac tgtcaatgca aaaggaataa tactatttat 600
agtattatac atcatcaca acatattcca aagacagaac agattactaa taggataaac 660
tatggaagac tatatattac atttcataaa ataaaaagct aagtgtgtta tttaaagggg 720
gtct 724

```

<210> 146  
 <211> 831  
 <212> DNA  
 <213> Gorilla

```

<400> 146
gtccttactt ccccatagaa atctagggcc tcttgtgcct ttaaaaattt gccccgatgt 60
aataaatatg cacaaatcat tacaccagtt cgctcccttc cagctttaca gtgaattgct 120
gcaacatgat tgctacttcc acttagccat tgggtcaagat cttcacaaaa gggtttgata 180
agttctagct gtggtgggtt atggtcttca aaaggatatt gtgcaactgt ggtaaaaaaga 240
taacctcaga ataagaaaaa aaactcctga atttttaatt aacaagtagg taactttaga 300
aatgctgcat acaaaactta caggtattta aaagaaacac tggattccag agaaaaataa 360
tgtattgctt aacttttctaa ttgttaaata gaaaacagtc tcttgataag tcttaaatat 420
aatcattaag gaagccaggt attattttcc cccattttat tcaggaggat atattctggg 480
aatttacgct atatggactg gtagcatagg tcacatatta gaggtagagc taaacccaaa 540
acgaactgtc acatggacat ttcgtcagga ctctcaatgc aaaaggaata atactattta 600
tagtatttat wtcacatca caaaacatat tccaaagaca gaatagatta ctaataggat 660
aaactatgca aagaactaca tattacatt cataaaaata aaatgctaag tgtgttattt 720
aaagggtggtc ttgcaaatgt tagtggttga tacacatgta atcattaggg aagccaagta 780
ttattttctc ccgttttctg caggagaata cattctggga atctatgctc a 831

```

<210> 147  
 <211> 556  
 <212> DNA  
 <213> Domestic pig

```

<400> 147
tctctgggtc ttacttcccc atagaaatct agggcctctt gtgcctttta aaattttacc 60
cgatgtaaca aatatgcaca aatcattaca ccagttcgtc cctttccagc tttacagtga 120

```

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attgctgcaa catgattgtc atcttcactt agccattggg caagatcttc acaaaaaggt 180
ttgataagtt cttagctgtg tggattatgg tcttcgaaaag gatactgtgc aactgtggaa 240
aaagataacc tcagaataaa aaaatctctc ctgagttgct aattaaaagt aggttaactt 300
ttgaaatctt gcatataaat tcaatagaga ttttaataaa aaactgaact ccagggaaaa 360
attgtctgat aattttcaaa tagaaaatag aaaataatct cctgttaact caaatttccc 420
cattagatag ggaggccaag tatcattttc cccactttat gaaggaggaa actttgcaat 480
agagtagcaa tgtatcagag gtcacaacgt atcagaaatg gaggtaaact caaaatgaaa 540
tgtcacatga gccctt 556
```

<210> 148  
<211> 752  
<212> DNA  
<213> Wild boar

```
<400> 148
tctctgggtcc ttacttcccc atagaaatct agggcctctt gtgcctttta aaatttaccc 60
cgatgtaaca aatatgcaca aatcattaca ccagttcgtc ctttccagc tttacagtga 120
attgctgcaa catgattgtc atcttcactt agccattggg caagatcttc acaaaaaggt 180
ttgataagtt cttagctgtg tggattatgg tcttcgaaaag gatactgtgc aactgtggaa 240
aaagataacc tcagaataaa aaaatctctc ctgagttgct aattaaaagt aggttaactt 300
ttgaaatctt gcatataaat tcaatagaga ttttaataaa aaactgaact ccagggaaaa 360
attgtctgat aattttcaaa tagaaaatag aaaataatct cctgttaact caaatttccc 420
cattagatag ggaggccaag tatcattttc cccactttat gaaggaggaa actttgcaat 480
agagtagcaa tgtatcagag gtcacaacgt atcagaaatg gaggtaaact caaaatgaaa 540
tgtcacatga gcccttctta tcagggttta ccatatattt tctaacaaaa ggagttgcag 600
tacttataat attggatcat tacaaaatgt atgtttcaaa gaaagtatag ttcactaata 660
aatcaacaat ggaaaagata gcaatttggt ctccatataa taaaaatgcc aagcatgtta 720
ttttaaagat ggtcttgcta atagtgtgt at 752
```

<210> 149  
<211> 715  
<212> DNA  
<213> Cattle

```
<400> 149
ctctgggtcct tacttcccc tagaaatcta gggcctcttg tgccctttaaa aatttgcccc 60
gatgtaacaa atatgcacaa atcattacac cagttcgtcc ctttccagct ttacagtga 120
ttgctgcaac atgattgtca tcttcactta gccattgggc aagatcttca caaaagggtt 180
tgataagttc taactgtggt ggattatggt ctccaagggt atactgtgca actgtgata 240
aaaaataacc tcagaataag aaaataatct cacttgaatt gcttattaca agtaggttaa 300
ctttagaaat gttgcataca aatagttaa aaatatctga actatagaga aaaagaattt 360
attgtctgat aattttctaa ttttgaacag aaaataatct ctcatnaact caaattttatc 420
cattagacag gtacgtcaag tattattttc ctactttat gatggaggca atggagtagc 480
aacatatacag aggtcacaac ataacagagg gagaggtaaa ctcaaaatga tacatcaca 540
gagcctctta tcagggtct caatacattt tctagcaaaa ggaactgtaa tatctataat 600
attgcattat cacaaaatat gtattccaaa gaaagcaaa atcctaataa atcacaatgc 660
aaagactgca ttttatgcta tatatacaga aggcagcata ttatttttaa gatgg 715
```

<210> 150  
<211> 708  
<212> DNA  
<213> Banting cattle

```
<400> 150
ggctccttact tccccataga aatctagggc ctcttggtgcc tttaaaaatt tgccccgatg 60
taacaaatat gcacaaatca ttacaccagt tcgtcccttt ccagctttac agtgaattgc 120
tgcaacatga ttgtcatctt cacttagcca ttggtcaaga tcttcacaaa aggggttgat 180
```

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```

aagtttctaac tgtggtggat tatggtcttc aaaggggatac tgtgcaactg tgataaaaaa 240
ataacctcag aataagaaaa taatctcact tgaattgctt attacaagta gggttaacttt 300
agaaatgttg catacaataa gtttaaaaaat atctgaacta tagagaaaaa gaattttattg 360
tctgataaatt ttctaatttt tgaacagaaa ataactcttc attaactcaa atttatccat 420
tagacaggta cgtcaagtat ttttttcctc actttatgat ggaggcaatg gagtagcaac 480
atatcagagg tcacaacata acagagggag aggtaaactc aaaatgatac atcacatgag 540
cctcttatca gggctctcaa tacattttct agcaaaagga actgtaatat ctataatatt 600
gcattatcgc aaaatatgta ttccaaagaa agcaaaagatc actaataaat caacaatgca 660
aaagactgca ttttatgcta tatatacaga aggcaagcat attattttt 708

```

<210> 151  
 <211> 548  
 <212> DNA  
 <213> Red buffalo

```

<400> 151
gggtccttact tccccataga aatctagggc ctcttgtgcc tttaaaaaatt ttccccgatg 60
taacaaatat gcacaaatca ttacaccagt tcgtcccttt ccagctttac agtgaattgc 120
tgcaacatga ttgtcatctt cacttagcca ttggtcaaga tcttcacaaa aggggtttgat 180
aagtttctaac tgtggtggat tatggtcttc aaaggggatac tgtgcaactg tgataaaaaa 240
ataacctcag aataagaaaa taatctcact tgaattgctt attacaagta gggttaacttt 300
agaaatgttg catacaaaaga gtttaaaaaat atctgaacta tagagaaaaa gaattttattg 360
tctgataaatt ttctaatttt gaacagaaaa taatctctca ttaactcaaa tttatccatt 420
agacaggtaa gtcaagtatt attttcctca ctttatgatg gaggcaatgg gtagcaacat 480
atcagaggca caacataaca gaggggaaaag gtaaaactcaa aatgaaacat cacatgagcc 540
tcttatca 548

```

<210> 152  
 <211> 700  
 <212> DNA  
 <213> Sheep

```

<400> 152
tctggctcctt acttccccat agaaatctag ggctcttgtt gcctttaaaa atttgccccg 60
atgtaacaaa tatgcacaaa tcattacacc agttcgtccc tttccagctt tacagtgaat 120
tgctgcaaca tgattgtcat cttcacttag ccattgggtca agatcttcac aaaagggttt 180
gataagttct aactgtgggtg gattatgggt ttcaaaggga tactgtgcaa ctgtgataaa 240
aagataaccg cagaataaga aaataatctc acttgaattg cttattacaa gtaggctaac 300
tttagaaaatg ttgcatacaa atagttttaa aatrtcttraa ctatagagga aaagaattta 360
ttgtctgata attttctaatt ttctgaacag aaaataatct ctcatttaact caaattttatc 420
cattcgacag gtaagacaag tattattttc ctactctat gatggaggga atggaggagc 480
aacatatcag aggtcacacac ataacggagg aagaggcaaa ctcagaatga aacgtcgcac 540
gagcctctta gcagggtctt caatacgttt cctagcaaaa ggaactgtaa catctataat 600
atcgcallat cacaaaacat gtattccaaa gaaagtacag atcactaata agtcaacaat 660
gcagaagact gcatttttatg cttgacgtga cagaaaggca 700

```

<210> 153  
 <211> 780  
 <212> DNA  
 <213> Bighorn

```

<400> 153
ccttacttcc ccatagaaat ctagggcctc ttgtgccttt aaaaatttgc cccgatgtaa 60
caaatalgca caaatcatta caccagttcg tccctttcca gctttacagt gaattgctgc 120
aacatgattg tcactctcac ttagccattg gtcaagatct tcacaaaagg gtttgataag 180
ttctaactgt ggtggattat ggtcttcaaa gggatactgt gcaactgtga taaaaagata 240
accgcagaat aagaaaaataa tctcacctga attgcttatt acaagtaggc taactttaga 300

```

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```

aatgttgcac acaaatagtt taaaaatatc tgaactatag tggaaaagaa tttattgtct 360
gataattttc taatttttgc acagaaaata atctctcatt aactcaaatt tatccattcg 420
acaggtaaga caagtattat tttcctcact ctatgatgga ggcaatggag gagcaacata 480
tcagaggtea cagcataacg gaggaagagg caaactcaga atgaaacgtc gcacgagcct 540
cttagcaggg ctctcaatac gtttcctagc aaaagggaact gtaacatcta taatatcgca 600
ttatcacaaa acatgtatcc caaagaaagt acagatcact aataagtcaa caatgcagaa 660
gactgcattt tatgcttgac gtgacagaaa gggcaagcat attattttaa gatgggtctcg 720
aaaatgcaac tgttgcgtag acacaattct aaagacattc acaaagacac ttaaaaaattt 780

```

<210> 154  
 <211> 463  
 <212> DNA  
 <213> Cameroon sheep

```

<400> 154
acttccccat agaaatctag ggctctttgt gcttttaaaa atttgccccg atgtaacaaa 60
tatgcacaaa tcattacacc agttcgtccc tttccagctt tacagtgaat tgctgcaaca 120
tgattgtcat ctccacttag ccattgggta agatcttcac aaaagggttt gataagttct 180
aactgtgggtg gattatgggc ttcaaaggga tactgtgcaa ctgtgataaa aagataaccg 240
cagaataaga aaataatctc acttgaattg cttattacaa gtaggcgggt ttagaaatgt 300
tgacatacaa tagtttaaaa atgtctgaac tatagaggaa agaatttatt gtctgataat 360
tttctaattt tcgaacagaa aataatctct cattaactca aatttatcca ttcgacaggt 420
agacaagtat tattttctca ctctwtgatg gaggcattgg agg 463

```

<210> 155  
 <211> 524  
 <212> DNA  
 <213> Deer

```

<400> 155
tctctgggtc ttacttcccc gtagaaatct agggcctctt gtgcctttta aaatttgccc 60
cgatgtaaca aatatgcaca aatcattaca ccagttcgtc cctttccagc tttacagtga 120
atcgtgtcaa catgattgtc atcttcactt agccattggg caagatcttc acaaaagggc 180
ttgataagtt ctaactgtgg tggattatgg tcttcaaagg gatactgtgc aactgtgata 240
aaaaaatgac ctccagaataa gaaaataatt tcacttgaat tgcttattac aagtaggtta 300
actttagaaa tgttgcatat aaataagtta aaaatatccg aaccataaag aaaaagaatt 360
tattgtctgg taattttcta atttttgaac agaaaataat ctctcattaa ctcaaattta 420
tccattagaa aggtaagtca agtattgttt tctcacttcc atgatggagg caatggagga 480
gcaacatata agaggcacag cataacagag gaagagggtaa actc 524

```

<210> 156  
 <211> 647  
 <212> DNA  
 <213> Roe deer

```

<400> 156
tctctgggtc ttacttcccc gtagaaatct agggcctctt gtgcctttta aaatttgccc 60
cgatgtaaca aatatgcaca aatcattaca ccagttcgtc cctttccagc tttacagtga 120
atcgtgtcaa catgattgtc atcttcactt agccattggg caagatcttc acaaaagggc 180
ttgataagtt ctaactgtgg tggattatgg tcttcaaagg gatactgtgc aactgtgata 240
aaaagataac ctccagaataa gaaaataatt tcacttgaat tgcttattac aagtaggtta 300
actttagaaa tgttgcatat aaataagtta aaaatatcca aaccataaag aaaaagaattt 360
attgtctgat aatttttcta tttttgaaca gaaaataatc tcttatwaac tcaaatgtat 420
ccattagaaa ggtaagcaga gtattgtttt cctcacttca tgatgcaggc aatggaggag 480
caacatatca gaggtcacag cataacagag gaagagggtta actcacaatg aaacatcaca 540
tagcctctta tcaggactct caatacattt tctagcagaa ggaaccgtta tatctataac 600

```



attgcattat cacaaagtat gtattccaaa taaagtlacat aacacta

647

<210> 157  
<211> 512  
<212> DNA  
<213> Goitred gazelle

<400> 157  
tccttacttc cccatagaaa tctagggcct cttgtgcctt taaaaatttg ccccgatgta 60  
acaaatatgc acaaatcatt acaccagttc gtccctttcc agctttacag tgaattgctg 120  
caacatgatt gtcattctta cttagccatt ggtcaagatc ttcacaaaag ggtttgataa 180  
gttctaactg tgggtggatta tggctttcaa agggatactg tgcaactgtg ataaaaagat 240  
aacctcagaa taagaaaata atctcacttg aattgcttat tataagtagg ttaactttat 300  
aaatgttgca tacaacaggt ttaaaaatat ctgaactaca gagaaaaaga atttattgtc 360  
tgataatttc taattttttg acagaaaata atctctcata actcaaattt acccattaga 420  
caggtaagcc aagtattatt ttctcacttt atgatggagg caatggagta gcacatatca 480  
gaggcacaac ctaacagagg agaggtaact ca 512

<210> 158  
<211> 798  
<212> DNA  
<213> Horse

<400> 158  
ggctccttact tctccataga aatctagggc ctctgtgtgc tttaaaaact tgccccgatg 60  
taacaaatat gcacaaatca ttacaccagt tgcgtccctt ccagctttac agtgaattgc 120  
tgcaacatga ttgtcatctt cacttagcca ttgggtcaaga tcttcacaaa aggggttgat 180  
aagttctagc tgtggtggat tatgatcttc aaaaggatac tgtgcaactg tggtaaaaag 240  
ataatctcaa attaagaaaa aaatctctcc tgaattgttt attaaaagta ggttaacttt 300  
aggaatgctg cgtataagtt taacagatat ttaaaagaaa aactgaactc cagagaaaaa 360  
taattttattg tctgataatt ttctaatttt tgaatagaaa ataagagtcc cattaattct 420  
caaaactcat ccattagaca gggaagccaa gtattatttt ccctactcta tgaaggagta 480  
cattgtgcta tgcagaggta gcaaagggtc caacacataa gacatggagg tgaactcaaa 540  
atgaaatgtc acatgggcct cttgttatgg ctttcaatgc atactctaac aaaaggagaa 600  
ataacactta gaattattgc tcaccacaaa acatatatct caaagaaagt acagattact 660  
aataaatcaa cgggaaggat ggcattttac acttcatata ataaaaatgc taactgtgtt 720  
atttttaaga tgggtctggca aatggtagcg ctgtataccg actttaacag cattttacaa 780  
gaaaactggaa aatcactt 798

<210> 159  
<211> 519  
<212> DNA  
<213> African elephant

<220>  
<221> misc\_feature  
<222> (1)...(519)  
<223> n = A,T,C or G

<400> 159  
tggtccttac ttcnnnnnnn nnnnnnnnnn nnncttgtgc ctttaaaaaat ttgccccgat 60  
gtaacaaata tgcacaaatc attacaccag ttogtccctt tccagcttta cagtgaattg 120  
ctgcaacatg attgtcatct tcacttagcc attgggtcaag atcttcacaa aagggtttga 180  
taagctctag ttgtggtggg ttgtggtctt caaaaggata ctgtgcaact gtggtaaaaa 240  
gataaactca gaataagaaa aaaatctctc ctgaattttt aattaaaagt aggttagctt 300  
cagaaacatt gcacataaac tataaacagg tgttttaata aaagataagc taaactccct 360  
taaaaaaaaaa tttattgcct gataaactgc tagtttttga atatagtctc tcaactaactc 420

ttaaatgcat ccattaaaaa aggagaccaa gtattatattt cccacatta tgctagagga 480  
aactgtgtta tgctgaagta gcacaggta catctcaga 519

<210> 160  
<211> 776  
<212> DNA  
<213> Indian elephant

<220>  
<221> misc\_feature  
<222> (1)...(776)  
<223> n = A,T,C or G

<400> 160  
tggctccttac ttccccataa aaatctaggg cttcttgtgc ctttaaaaaat ttgccccgat 60  
gtaacaaata tgcacaaatc attacaccag ttctgccctt tccagcttta cagtgaattg 120  
ctgcaacatg attgtcatct tcacttagcc attgggtcaag atcttcacaa aagggtttga 180  
taagctctag ttgtgggtggg ttgtgggtctt caaaaggata ctgtgcaact gtggtaaaaa 240  
gataaactca gaataagaaa aaaatctctc ctgaattttt aattaaaagt aggttagctt 300  
cagaaacatt gcacataaac tataaacagg tgttttaata aaagataagc taaactccat 360  
taaaaaaaaaa ttatttgctt gataacttgc tagtttttga atatagtctc tcactaactc 420  
ttaaatgcat ccattaaaaa aggagaccaa gtattatattt cccacatta tgctagagga 480  
aactgtgtta tgctgaagta gcacaggta catctcagag gtggagctga acccaaaaag 540  
aaatgtttaca taggcctctt gtcaagggtc gtcaatgcat tttctaacia aaggagtagt 600  
gacactaata atattgcac accttggtaa cagaacatat tctcaaaggc agaattgatt 660  
attaacagaa tcagtaattg aaaggattgc attttatact tcatataaaa natgttcggt 720  
ctattatttta aagggtggctt lacaaatggt agtggtgtat acaatgattt ataaga 776

<210> 161  
<211> 701  
<212> DNA  
<213> Dog

<400> 161  
ggtccttact tccccataga aatctagggc ctcttgtgac tttagaaatt tgccccgatg 60  
taataaatat gcacaaatca ttacaccagt tctgcccttt ccagctttac agtgaattgc 120  
tgcaacatga ttgtcatctt cacttagcca ttgggtcaaga tcttcacaaa aagggtttgat 180  
aagttctagc tgtgggtggat tatgggtctt caaaaggatac tgtgcaactg tggtaaaaaa 240  
ataacctcag aattagaaaa aagtcctttc tgaactgttt attaaaagta ggtaactttt 300  
agaaacattg catgtaagct taacagatgt ttaaaagaaa aacggaactc cagagaaaaa 360  
taattttgctg tctgataatt ttccaatttt tgaatagaaa atagtctctc attaatctct 420  
aaacctacca ctagagagag aggctaagca ttattttccc cactttaatg aaagaggaaa 480  
ctttgcaatg gagaggggagc acacgtcaac atatcagagg gaagaggcaa actcaaaatg 540  
aaatggcaca caggttttctt gtcagggtct tcaatgcatt ttctgacaaa aggagtcata 600  
atattttataa tactacgtca tcacaaaata tatattccag agaaagtata aataaccgat 660  
aatcaatga tggaaaggat tgattttaca cttgatataa t 701

<210> 162  
<211> 603  
<212> DNA  
<213> Sun bear

<220>  
<221> misc\_feature  
<222> (1)...(603)  
<223> n = A,T,C or G

```
<400> 162
ggtccttact tennnncata gaaatctagg gcctcttgtg cctttaaaaa tttgccccga 60
tgtaataaat atgcacaaat cattacacca gtctgtccct ttccagcttt acagtgaatt 120
gctgcaacat gattgtcatc ttcaacttagc catttggtcaa gatcttcaca aaaggggttg 180
ataagttcta gctgtggttg attatggtct tcaaaaggat actgtgcaac tgtggtaaaa 240
ggataacctc agaattagaa aaaagtcttt cctgaattgt ttattaaaga aggttaactt 300
tagaaatggt gcatataagc ttaacagatg tttaaaagaa aaactaaact ccagagaaaa 360
taatttgctg cctgacaatt tacgaatttt tgaatagaaa acagtctctc attaatctct 420
aaacccaccc acaagacaga ggccaagcat tatgttcccc acttaactga agaggaaaga 480
aactttgcta tggagaggta gcacaagtca catatcagag ggagaggcaa attcnaaatg 540
aaatgtcacg taggtagggt tctgttaggg ctctcaatgc atttttctga caaaaggagt 600
cgt 603
```

```
<210> 163
<211> 536
<212> DNA
<213> Mouse
```

```
<400> 163
ccttacttcc ccataaaaaat ctagggcctc ttgtgccttt aaaaatttgc cccgatgcaa 60
taaatatgca caaatcatta caccagtcog tccctttcca gctttacagt gaattgctgc 120
aacatgattg tcatcttcac ttageccattg gtcaagatct tcacagaagg gtttgataag 180
ttctagctgt ggtgggttat ggtcttcaaa aggatactgt gcaactgttg caaaaagata 240
atcccagtgat aagaaaaattt taaatttttt atttaaaaac ataggttaac tttcaaaatg 300
ttatatataa acttactggt tcttaaaaga agcctaactt tcaggaaatt ttaattttatt 360
actaattaaa cctagatttt aaagaaagtc ttttattaat tcttaaatgc attcattaga 420
catggaaaca agcattgtgc tcttcaactcc agggaggatg aatctgtgca tgaagggaac 480
acgtcatagc ctatcagtc cactgaatcca aatgcacgct acccaggcac ttgtca 536
```

```
<210> 164
<211> 696
<212> DNA
<213> Guinea pig
```

```
<400> 164
acttctccat agaaatctag agcctcttgt gcctttaaaa atttgccccg atgtaataaa 60
tatgcacaaa tcattacacc agtccgtccc ttccagctt tacagtgaat tgctgcaaca 120
tgattgtcat cttcaacttag ccattgggtca agatcttcac aaaaagggtt gataagttct 180
agctgtggtg ggttatgatc ttcaaaaaggg tattgtgcaa ctgtgataaa aacataatct 240
cagagtaaga aagggatctt gcctaaattt ctaatcagaa ataggtaaac tttagaaatg 300
tttcacataa actcaagatg tttaaacaga aaaactgaac tgcatagaaa aataattttat 360
tgttcgttta cttttttact ttcttttttt aaaatacaaa atagtctatt agtaactttt 420
aaacgtatct attacacaag gtggccaggt attacgttct tcaacttcacg caggagaaaa 480
ctgtgatttg acagggaaca cagatcataa aacatcaaaag atacatcgaa tccaaaaaaa 540
taccaggtca cacagcctct cataacgtct ttagggtgaat ttctgacaaa agcagtaaca 600
tttattatac tgcataacca tacaacacac tttgaaggaa gtatgaacta ctaatrggat 660
acactatgaa aaarmtgcac tttatatttt ataaat 696
```

```
<210> 165
<211> 695
<212> DNA
<213> Himalaya-Tahr
```

```
<220>
<221> misc_feature
<222> (1)...(695)
<223> n = A,T,C or G
```

```
<400> 165
acttcnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnna atttgccccg atgtaacaaa 60
tatgcacaaa tcattacacc agttcgcccc ttccagctt tacagtgaat tgctgcaaca 120
tgattgtcat cttcacttag ccattgggtc agatcttcac aaaagggttt gataagttct 180
aactgtgggtg gattatgggtc ttcaaaggga tactgtgcaa ctgtgataaa aagataaccg 240
cagaataaga aaataatctc acttgaattg cttattacaa gtaggttaac tttagaaatg 300
ttgtatacaa atagttttaa aatatctgaa ctatagagga aaagaattta ttgtctgata 360
atcttctaata tttgaacaga aaataatctc tcattaactc aaatttatcc attcgacagg 420
taagacaagt attcttttcc tcaactctatg atggaggcaa tggaggagca acatatcaga 480
ggtcacaaca taacgsagga agaggcaaac tcaagagtga aacgtcgcac gagcctctta 540
tcaggcctct ccaatacgtt tcctagcaaa aggaactgta acatctataa tategcatta 600
tcacaaaaca tgtattccaa agaaagtaca gatcactaat aggtccaatg cagaagactg 660
cattttatgt tgatgtgaca gaaaggcaaa gcata 695
```

```
<210> 166
<211> 281
<212> DNA
<213> Human
```

```
<400> 166
ccttacttcc ccatagaaat ctagggcctc ttgtgccttt aaaaatttgc cccgatgtaa 60
taaatatgca caaatcatta caccagttcg tccctttcca gctttacagt gaattgctgc 120
aacatgattg tcattcttcac ttagccattg gtcaagatct tcacaaaagg gtttgatcag 180
ttctagctgt ggtgggttat ggtcttcaaa aggatattgt gcaactgtgg taaaaagata 240
acctcagaat aagaaaaaaa actcctgaat ttttaattac a 281
```

```
<210> 167
<211> 373
<212> DNA
<213> Vikunja
```

```
<220>
<221> misc_feature
<222> (1)...(373)
<223> n = A,T,C or G
```

```
<400> 167
ccttacttcc nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnngatgtaa 60
caaatatgca caaatcatta caccagttcg tccctttcca gctttacagt gaattgctgc 120
aacatgattg tcattcttcac ttagccattg gtcaagatct tcacaaaagg gtttgataag 180
ttctagctgt ggtggattat ggtcttcaaa aggatactgt gcaactgtgg taaaaaaaaa 240
agaaaagaaa aaaagaacct cagaataaga aaaaaaatct cccctgaact gcttattaaa 300
tgcaagttaa ctttggaat gttggcatat taaccttaac agacgtttta aaaggaaaat 360
ctgaactcca gag 373
```

```
<210> 168
<211> 291
<212> DNA
<213> Spotted mustang
```

```
<220>
<221> misc_feature
<222> (1)...(291)
<223> n = A,T,C or G
```

```
<400> 168
ctctgtcct tacttcccca tagaaatcta gggcctcttg tgcctttaaa aatttgcccc 60
```

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gatgnaataa atatgcacaa atcattacac cagttcgtcc ctttccagct ttacagttaa 120  
 ttgctgcaac atgattgtca tcttcaactga gccattgggc aagatcttca caaaagggtt 180  
 tgataagttc cagctgcggg ggggttatggg cttcaaaagg atactgtgca actgtgtaaa 240  
 aagatcacct cagagtgaaga aaagagtcct tctgaactg tttcttaaaa g 291

<210> 169  
 <211> 598  
 <212> DNA  
 <213> Fishing cat

<400> 169  
 acttccccat agaaatctag ggctcttctg gccttttaaaa atttgccccg atgcaataaa 60  
 tatgcacaaa tcattacacc agttcgtccc tttccagctt tacagtgaat tgctgcaaca 120  
 tgattgtcat cttcaactgag ccattgggtc agatcttcac aaaagggttt gataagttcc 180  
 agctgcggtg ggttatgggc ttcaaaagga tactgtgcaa ctgtgtaaaa agatcacctc 240  
 agaattgagaa aagaggcctt cctgaattgc ttcttaaaag taggttaact tcagaaacgt 300  
 tgcataataag ctttaacagat gtttagaagg aaaactaaac tccagagaaa aatactcgtc 360  
 tgatgatttt ccaatttttg aacagaaaac agtctctcat taatttttaa acctatgcac 420  
 tagacagaga ggccgattat ttccccccat gacgaagagg agactgctct ggagagcaag 480  
 cacaagtcac aacgtgtcag agggagagga ggacccggaa tgtcacacag gtttctgtgc 540  
 agggctctca atgcattttc tgacaaaatg agtaatacgc ttatactatt acatcatc 598

<210> 170  
 <211> 220  
 <212> DNA  
 <213> Turkey

<220>  
 <221> misc\_feature  
 <222> (1)...(220)  
 <223> n = A,T,C or G

<400> 170  
 ctctggctct tacttcccca tagaaatcta gggcttcttg agcctttaaa aatttgccctc 60  
 gatgtaataa atatgcacat atcattacac cagttcgtcc ctttccagct ttacagttaa 120  
 ttgctgcaac atgattgtca tcttcaactta gccattgggc aagatcttca caaaagggtt 180  
 tgataagctc taactgtggg ggggttatggg cttcaaaagg 220

<210> 171  
 <211> 505  
 <212> DNA  
 <213> Cockerel

<220>  
 <221> misc\_feature  
 <222> (1)...(505)  
 <223> n = A,T,C or G

<400> 171  
 tctggctcct acttccccat agaaatctag ggcttcttga gccttttaaaa acttgccctg 60  
 atgcaacaaa tatgcacata tcattacacc agttcgtccc tttccagctt tacagtggat 120  
 tgctgcaaca tgattgtcat cttcaacttag ccattgggtc agatcttcac aaaaagggtt 180  
 gataagctct aactgtggg ggttatgggc ttcaaaaggg tactgtgcaa ctgtaatgag 240  
 aaggattaac ttattaaaat ctaaaaagga taatcaccaa gagctcaact agacagggtc 300  
 aatttgtgac aagcatgaaa aaattaacat tctaaatata gtcttgcata tagatttgta 360  
 tacacgcaac tttgattctg ctgttatttc gtaacattgc acactaaatg catcacaaat 420  
 ttctctagta atacgtaagt atcttactgg catgaagagg actatcccac cgtgcttctg 480

nagtttntac tacagactct gcacc

505

<210> 172  
<211> 645  
<212> DNA  
<213> Duck

<220>  
<221> misc\_feature  
<222> (1)...(645)  
<223> n = A,T,C or G

<400> 172  
ccttacttcc ccatagaaat ctagagcttc ttgagccttt aaaaacttgc ctctatgcaa 60  
cagatatgcg catatcatta caccagttcg tccctttcca gctttacagt ggattgctgc 120  
aacatgattg tcatcttcac ttagccattg gtcaagatct tcacaaaaag gtttaatgag 180  
ctcaagctgt ggtgggttat ggtcttcaaa aggggtactgt gcaactgcaa caagaaagaa 240  
aaacttacca aaatctcaaa aggaaactac agcaagcttg actagacgtg tcatctttgg 300  
acaagcacac acaaaaatta acattctaaa taaaaactgt cttatatgta tatacatata 360  
gctttgcttt cactgttatt cagcagcata ctatacactn ttncacatca cagacatttc 420  
tctattaata cataagcaca tatcttagac tatttcacag tgcttctgaa acaagtgcga 480  
cagactctat tttaacttat ttttctgaaa tttaagagtgt cactggcaca aagaataacc 540  
ttgtgaaaac ccattagtca cagactacct gctgagagaa agcagggcaa acctcactca 600  
ctgatcagag acaggggattt tgcagcaaga attctgagtg gctgg 645

<210> 173  
<211> 516  
<212> DNA  
<213> Quail

<220>  
<221> misc\_feature  
<222> (1)...(516)  
<223> n = A,T,C or G

<400> 173  
ccttacttcc nnnnnnnnnn nnnnnnnnnn nnnnnccttt aaaaacttgc ntcatgcaa 60  
caaatatgca catatcatta caccagttcg tccctttcca gctttacaat ggattgctgc 120  
aacatgattg tcatcttcac ttagccattg gtcaagatct tcacaaaaag gtttgataag 180  
ctctagctgt ggtgggttat ggtcttcaaa aggggtactgt gcaactgcaa tgagaaggaa 240  
taacgttcta aataaaacac agtcttgcat acagatttgc atccacacag ctttgattct 300  
gttggttattc agcagcatat tacacactat aaatgcatca catgtttctc tagtaatacg 360  
taagcatctt gctgcatgaa gagacctcag aagcattgtg ggaatagtta gtgctaccaa 420  
ctgcacctta caccatgatt ttactcaaat taagagtgtg ctggcacaac aaataacgtg 480  
ttttaaggtc acccatcaaa tgcagtgctt tttttt 516

<210> 174  
<211> 395  
<212> DNA  
<213> Trout

<220>  
<221> misc\_feature  
<222> (1)...(395)  
<223> n = A,T,C or G

<400> 174

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```
tctctgggtcc ttacttcennn nnnnnnnnnn nnnnnnnnnn ngctttgagg aacttgcccc 60
ggtgtaacag gtaagcacag atcatgacac ccgtacgtcc ctttccagct ttacagtga 120
tcgcccgcac atgattgtcg tcttcaacta accaaaggct aagatcttcg cagaacgggt 180
tgatcagctc cagctggggc ggattgtgat cctcaaacgg atattgtgca actggagana 240
gacagacaga gaccggggtc agtttagttag cgtcacacgt gggtttttag tgaaagattg 300
attcattcac tgactgcctg aaagacagtg ataatggttt cactctgatg taatatctaa 360
cctctgcaat tgaatttggt ttgcgtcata atgtc 395
```

<210> 175  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTENse sense

<400> 175  
atcttgacca atggctaagt g 21

<210> 176  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Zoo44aRV

<400> 176  
ttgtctctgg tccttacttc 20

<210> 177  
<211> 160  
<212> DNA  
<213> Goat

```
<400> 177
tctctgggtcc ttacttcccc atagaaatct agggcctctt gtgcctttta aaatttgccc 60
cgatgtaaca aatatgcaca aatcattaca ccagttcgct ctttccagc tttacagtga 120
attgctgcaa catgattgtc atcttcaact agccattggt 160
```

<210> 178  
<211> 150  
<212> DNA  
<213> Antelope

<220>  
<221> misc\_feature  
<222> (1)...(150)  
<223> n = A,T,C or G

```
<400> 178
ctggctctta cttccccata gaaatctagg gctnntgtg cttttaaaaa tttgccccga 60
tgtaacaaat atgcacaaat cattacacca gttcgtcctt ttccagcttt acagtgaatt 120
gctgcaacat gattgtcatc ttcacttagc 150
```

<210> 179  
<211> 153

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&lt;212&gt; DNA

&lt;213&gt; Kangaroo

&lt;400&gt; 179

```
tctctgtgcc ttacttcccc atagaaatct agagcctctt gtgcctttaa aaactttcct 60
cgatgtaata aatatgcaca aatcattacg ccagttcgtc cctttcctgc ttacagtga 120
attgctgcaa catgattgtc atcttcactt agc 153
```

&lt;210&gt; 180

&lt;211&gt; 154

&lt;212&gt; DNA

&lt;213&gt; Rabbit

&lt;400&gt; 180

```
gtctctggtc cttacttctc cataaaaaatc tagggcttct tgtgccttta aaaatttgcc 60
ccgatgtaat aatatgcac aaatcattac accagttcgt ccctttccag cttacagtgc 120
aattgctgca acatgattgt catcttcact tagc 154
```

&lt;210&gt; 181

&lt;211&gt; 155

&lt;212&gt; DNA

&lt;213&gt; Hare

&lt;400&gt; 181

```
ggtccttact tctccataaa aatctagggc ttcttgtgcc tttaaaaatt tgccccgatg 60
taataaatat gcacaaatca ttacaccagt togtcccttt ccagctttac agtgaattgc 120
tgcaacatga ttgtcatctt cacttagcca ttggc 155
```

&lt;210&gt; 182

&lt;211&gt; 159

&lt;212&gt; DNA

&lt;213&gt; Goose

&lt;400&gt; 182

```
tctctgtgcc ttacttcccc atagaaatct agagcctctt gagcctttaa aaacttgctc 60
cgatgcaaca aatatgcgca tatcattaca ccagttcgtc cctttccagc ttacagtgg 120
attgctgcaa catgattgtc atcttcactt agccattgg 159
```

&lt;210&gt; 183

&lt;211&gt; 156

&lt;212&gt; DNA

&lt;213&gt; Ostrich

&lt;400&gt; 183

```
ctctggctct tacttcccca tagaaatcta gggcttcctg agcccttaaa aacttgctc 60
gatgtaacaa ataagcacat atcattacac cagttcgctc cttccagct ttacagtgg 120
ttgctgcaac gtgattgtca tcttcactta gccatt 156
```

&lt;210&gt; 184

&lt;211&gt; 151

&lt;212&gt; DNA

&lt;213&gt; Pigeon

&lt;400&gt; 184

```
tctggctcctt acttctccgt agaaatctag ggcttcttga gcctttaaaa acttgctcgt 60
atgcaacaaa tatgcacata tcattacacc agttcgctcc tttccagctt tacagtggat 120
tgctgcaacg tgattgtcgt cttcacttag c 151
```



012627-025.ST25

<210> 185  
<211> 163  
<212> DNA  
<213> Varan

<400> 185  
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cgatgtaata aatatgcaca aatcattaca ccagttcgtc cttttccagc ttacaatgg 120  
attgccgcaa cgtgattgcc atcttcactt agccattggt caa 163

<210> 186  
<211> 160  
<212> DNA  
<213> Trout

<400> 186  
tctgggtcctt acttcaccgt agaagtcacg agcttctctg gctttgagga acttgccccg 60  
gtgtaacagg taagcacaga tcatgacacc cgtacgtccc ttccagctt tacagtgaat 120  
cgccgccacg tgattgtcgt cctcacttag ccattgggtca 160

<210> 187  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTENex6F sense

<400> 187  
ggagtaacta ttcccagtc gag 23

<210> 188  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTENex6R antisense

<400> 188  
gcaagttccg ccactgaa 18

<210> 189  
<211> 138  
<212> DNA  
<213> Man

<400> 189  
ggagtaacta ttcccagtc gaggcgctat gtgtattayt atagctacct gktaaagaat 60  
catctggatt atagaccagt ggcactgttg ttccacaaga tgatgtttga aactattcca 120  
atgttcagtg gcggaact 138

<210> 190  
<211> 131  
<212> DNA  
<213> Chimpanzee

<400> 190  
ctattcccag tcagaggcgc tatgtgtatt attatagcta cctgttaaag aatcatctgg 60  
attatagacc agtggcactg ttgtttcaca agatgatgtt tgaaactatt ccaatgttca 120  
gtggcggaac t 131

<210> 191  
<211> 128  
<212> DNA  
<213> Cattle

<400> 191  
ttcccagtca gaggcgctat gtgtattatt atagctacct gttaaagaat catctggatt 60  
atagaccagt ggcactgttg ttccacaaga tgatgtttga aactattcca atgttcagtg 120  
gcggaact 128

<210> 192  
<211> 128  
<212> DNA  
<213> Sheep

<400> 192  
ttcccagtca gaggcgctat gtgtattatt atagctacct gttaaagaat catctggatt 60  
acagaccagt ggcactgttg ttccacaaga tgatgtttga aactattccc atgttcagtg 120  
gcggaact 128

<210> 193  
<211> 126  
<212> DNA  
<213> Goat

<400> 193  
tcccagtcag aggcgctatg tgtattatta tagctacctg ttaaagaatc atctggatta 60  
cagaccagtg gcactgttgt ttccacaagat gatgtttgaa actattccaa tgttcagtgg 120  
cggaac 126

<210> 194  
<211> 131  
<212> DNA  
<213> Red buffalo

<400> 194  
gtaactattc ccagtcagag gcgctatgtg tattattata gctacctgtt aaagaatcat 60  
ctggattata gaccagtggc actgttgttt cacaagatga tgtttgaaac tattccaatg 120  
ttcagtggcg g 131

<210> 195  
<211> 127  
<212> DNA  
<213> Deer

<400> 195  
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atagaccagt ggcactgttg ttccacaaga tgatgtttga aactattcca atgttcagtg 120  
gcggaac 127

<210> 196  
<211> 131

<212> DNA

<213> Roe deer

<400> 196

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attatagacc agtggcactg ttgtttcaca agatgatgtt tgaaactatt ccaatgttca 120  
gtggcggaac t 131

<210> 197

<211> 126

<212> DNA

<213> Goitred gazelle

<400> 197

cccagtcaga ggcgctatgt gtattattat agctacctgt taaagaatca tctggattat 60  
agaccagtgg cactgttgtt tcacaagatg atgtttgaaa ctattccaat gttcagtggc 120  
ggaact 126

<210> 198

<211> 132

<212> DNA

<213> Horse

<400> 198

actattccca gtcagaggcg ctatgtgtat tattatagct acctgttaaa gaatcatctg 60  
gattatagac cagtggcact gttgtttcac aagatgatgt ttgaaactat tccaatgttc 120  
agtggcggaa ct 132

<210> 199

<211> 125

<212> DNA

<213> Dog

<400> 199

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tagaccagtg gcactgttgt ttccacaagat gatgtttgaa actattccaa tgttcagtgg 120  
cgaa 125

<210> 200

<211> 129

<212> DNA

<213> Sun bear

<400> 200

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attatagacc agtggcactg ttgtttcaca agatgatgtt tgaaactatt ccaatgttca 120  
gtggcgga 129

<210> 201

<211> 128

<212> DNA

<213> Rabbit

<400> 201

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attatagacc agtggcactg ttgtttcaca agatgatgtt tgaaactatt ccaatgttca 120  
gtggcgga 128

<210> 202  
<211> 128  
<212> DNA  
<213> Hare

<400> 202  
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ttatagacca gtggcactgt tgtttcaca gatgatgttt gaaactattc caatgttcag 120  
tgccggaa 128

<210> 203  
<211> 127  
<212> DNA  
<213> Antelope

<400> 203  
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tatagaccag tggcactgtt gtttcacaag atgatgtttg aaactattcc aatgttcag 120  
ggcggaa 127

<210> 204  
<211> 127  
<212> DNA  
<213> Kangaroo

<400> 204  
tcccagtcag aggcgctatg tgtattacta tagccacctg ttaaagcacc atttgatta 60  
cagaccagtg gccctgctgt ttcacaagat gatgtttgaa acaattccaa tgttcagtgg 120  
cggaact 127

<210> 205  
<211> 133  
<212> DNA  
<213> Python

<400> 205  
actattccca gtcagagacg ctatgtatat tattatagct acctgttaaa gaatcatctg 60  
gattacagac cagtagcact gctgtttcat aaaatgatgt ttgaaacaat tccaatgttc 120  
agtggcggaa ctt 133

<210> 206  
<211> 132  
<212> DNA  
<213> Varan

<400> 206  
actattccca gtcagaggcg ctatgtatat tattacagct accttttaaa gaatcatctg 60  
gattacagac ccgtggcatt gctcttccat aaaatgatgt ttgaaacaat tccaatgttc 120  
agtggcggaa ct 132

<210> 207  
<211> 132  
<212> DNA  
<213> Turkey

<400> 207  
actattccca gtcagagacg ctacgtgtac tactatagct acctgttaaa gaatcacctt 60

gattacagac cagtggcact gctgtttcac aagatgatgt ttgaaacaat tcccatgttc 120  
agtggcggaa ct 132

<210> 208  
<211> 124  
<212> DNA  
<213> Chicken

<400> 208  
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cagaccagtg gcaactgctgt ttcacaagat gatgtttgaa acaattccca tgttcagtgg 120  
cgga 124

<210> 209  
<211> 127  
<212> DNA  
<213> Duck

<400> 209  
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cagaccagtg gcaactgctgt ttcacaagat gatgtttgaa acaattccca tgttcagtgg 120  
cggaact 127

<210> 210  
<211> 131  
<212> DNA  
<213> Quail

<400> 210  
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gtggcggaaac t 131

<210> 211  
<211> 130  
<212> DNA  
<213> Goose

<400> 211  
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ttacagacca gtggcactgc tgtttcacaa gatgatgttt gaaacaattc ccatgttcag 120  
tggcggaaact 130

<210> 212  
<211> 128  
<212> DNA  
<213> Ostrich

<400> 212  
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tacagaccag tggcactgct gtttcacaag atgatgtttg aaacaattcc aatgttcagt 120  
ggcggaaac 128

<210> 213  
<211> 126  
<212> DNA  
<213> Pigeon

<400> 213  
cccagtcaga ggcgctacgt gtattactat agctatctgt taaagaacca cctggattac 60  
agaccagtgg cactgctgtt tcacaagatg atgtttgaaa caattcccat gttcagtggc 120  
ggaact 126

<210> 214  
<211> 130  
<212> DNA  
<213> Trout

<220>  
<221> misc\_feature  
<222> (1)...(130)  
<223> n = A,T,C or G

<400> 214  
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ggcggaactt 130

<210> 215  
<211> 122  
<212> DNA  
<213> Carp

<400> 215  
gtcagaggcg atatgtgtac tactatagct accttctgaa gaataagctg gagtacaac 60  
ctgtggcctt gctctttcac aagatgggtgt ttgagacagt gcccatgttc agtggcgga 120  
ct 122

<210> 216  
<211> 130  
<212> DNA  
<213> Salmon

<400> 216  
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gtacaaacca gtggctctgc tgtccacaa gatgggtgttc gagacgggtgc ccatgttcag 120  
tggcggaact 130

<210> 217  
<211> 132  
<212> DNA  
<213> Wels

<400> 217  
actattccca gtcagaggcg atatgtgtac tactatagct accttctgaa gaataagctg 60  
gagtacaac ctgtggcctt gctctttcac aagatgggtgt ttgagacagt gcccatgttc 120  
agtggcgga ct 132

<210> 218  
<211> 129  
<212> DNA  
<213> Tench

<400> 218  
attcccagtc agaggcgata tgtgtactac tatagctacc ttctgaagaa taagctggag 60

tacaaacctg tggccttgct ctttcacaag atgggtgttg agacagtgcc tatgttcagt 120  
ggcggaact 129

<210> 219  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTENex7F sense

<400> 219  
cctcagtttg tggctcgcca 20

<210> 220  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTENex7R antisense

<400> 220  
ccttttttag catcttggtc tgttt 25

<210> 221  
<211> 168  
<212> DNA  
<213> Man

<220>  
<221> misc\_feature  
<222> (1)...(168)  
<223> n = A,T,C or G

<400> 221  
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cacgacggga agacaagttc atgtaytttg agttccctca gccgttacct gtntgtggtg 120  
atatcaaagt agagttcttc cacaacaga acaagatgct aaaaaagg 168

<210> 222  
<211> 159  
<212> DNA  
<213> Chimpanzee

<400> 222  
agtttgtggt ctgccagcta aaggtgaaga tatattcctc caattcagga cccacacgac 60  
gggaagacaa gttcatgtac tttgagttcc ctccagccgtt acctgtgtgt ggtgatatca 120  
aagtagagtt cttccacaaa cagaacaaga tgctaaaaa 159

<210> 223  
<211> 161  
<212> DNA  
<213> Cattle

<400> 223  
cagtttgtgg tctgccagct aaaggtgaag atatattcct ccaattcagg acccacacga 60

cggaagaca agttcatgta ctttgagttc cctcagccat tgctgtgtg tggtagacac 120  
aaagtagagt tctccacaa acagaacaag atgctaaaaa a 161

<210> 224  
<211> 160  
<212> DNA  
<213> Sheep

<400> 224  
gtttgtggtc tgccagctaa aggtgaagat atatctctcc aattcaggac ccacacgacg 60  
ggaagacaag ttcatgtact ttgagttccc tcagccgtg cctgtgtgtg gtgacatcaa 120  
agtagagttc tccacaaac agaacaagat gctaaaaaag 160

<210> 225  
<211> 161  
<212> DNA  
<213> Goat

<400> 225  
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cggaagaca agttcatgta ctttgagttc cctcagccgt tgctgtgtg tggtagacac 120  
aaagtagagt tctccacaa acagaacaag atgctaaaaa a 161

<210> 226  
<211> 153  
<212> DNA  
<213> Red buffalo

<400> 226  
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gggaagacaa gttcatgtac ttgagttcc ctcagccgtt gcctgtgtg ggtgacatca 120  
aagtagagtt cttccacaa cagaacaaga tgc 153

<210> 227  
<211> 159  
<212> DNA  
<213> Deer

<400> 227  
cagtttgtgg tctgccagct aaaggtgaag atatattcct ccaattcagg acccacacga 60  
cggaagaca agttcatgta ctttgagttc cctcagccgt tgctgtgtg tggtagacac 120  
aaagtagagt tctccacaa acagaacaag atgctaaaa 159

<210> 228  
<211> 162  
<212> DNA  
<213> Roe deer

<400> 228  
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cggaagaca agttcatgta ctttgagttc cctcagccgt tgctgtgtg tggtagacac 120  
aaagtagagt tctccacaa acagaacaag atgctaaaaa ag 162

<210> 229  
<211> 161  
<212> DNA  
<213> Goitred gazelle



<400> 229

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cggaagata agttcatgta ctttgagttc cctcagccgt tgcctgtgtg tgggtgacatc 120  
aaagtagagt tcttccacaa acagaacaag atgctaaaaa a 161

<210> 230

<211> 162

<212> DNA

<213> Horse

<400> 230

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acggaagac aagttcatgt actttgagtt cctcagccg ttgcctgtgt gtgggtgacat 120  
caaagtagag ttcttccaca aacagaacaa gatgctaaaa aa 162

<210> 231

<211> 162

<212> DNA

<213> Dog

<400> 231

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acggaagac aagttcatgt actttgagtt cctcagcca ttgcctgtgt gcgggtgacat 120  
caaagtagag ttcttccaca aacagaacaa gatgctaaaa aa 162

<210> 232

<211> 161

<212> DNA

<213> Sun bear

<400> 232

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cggaagaca agttcatgta cttcgagttc cctcagccgt tacctgtgtg tgggtgacatc 120  
aaagtagagt tcttccacaa acagaacaag atgctaaaaa a 161

<210> 233

<211> 162

<212> DNA

<213> Rabbit

<400> 233

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cggaagaca agttcatgta cttcgagttc cctcagccgt tgcctgtgtg tgggtgacatc 120  
aaagtagagt tcttccacaa acagaacaag atgctaaaaa ag 162

<210> 234

<211> 156

<212> DNA

<213> Hare

<400> 234

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tcaaagtaga gttcttccac aaacagaaca agatgc 156

<210> 235

<211> 160



<210> 240  
<211> 163  
<212> DNA  
<213> Quail

<400> 240  
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acgtgaagac aagtatatgt actttgaatt ccctcaacct ttgccggtat gcggtgatat 120  
caaagtggag ttcttccaca aacagaacaa gatgctaaaa aag 163

<210> 241  
<211> 160  
<212> DNA  
<213> Ostrich

<400> 241  
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tgaagacaag tatatgtact ttgaattccc tcaacccttg ccggtatgcg gtgatataaa 120  
agtgaattc ttccacaaac agaacaagat gctaaaaaag 160

<210> 242  
<211> 145  
<212> DNA  
<213> Pigeon

<400> 242  
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acgtgaagac aagtatatgt actttgaatt ccctcaacct ttgccggtat gcggtgatat 120  
caaagtggaa ttttccaca aacag 145

<210> 243  
<211> 163  
<212> DNA  
<213> Carp

<220>  
<221> misc\_feature  
<222> (1)...(163)  
<223> n = A,T,C or G

<400> 243  
tcagtttgtg gtctgccaac tgaagggtgaa aatccacacc tcaaaccagc ygcacacaag 60  
gagagaggag aagtacatgt acttngattt tccncagcnn ctgcctgtgt gnggagacat 120  
caaggtggag ttcttccaca aacagaacaa gatgctaaaa aag 163

<210> 244  
<211> 160  
<212> DNA  
<213> Wells

<220>  
<221> misc\_feature  
<222> (1)...(160)  
<223> n = A,T,C or G

<400> 244  
agtttgtggt ctgccaactg aaggtgaaaa tccacacatc aaaccagng cacacaaggc 60

gagaggagaa gtacatgtac ttngattttc cncagcnnct gcctgtgtgn ggagacatca 120  
aggtggagtt cttccacaaa cagaacaaga tgctaaaaaa 160

<210> 245  
<211> 159  
<212> DNA  
<213> Tench

<400> 245  
agtttgtggt ctgccagctg aaggtgaaaa tccacacctc caaccacagc cacacaaggc 60  
gagaggagaa atacatgtac ttogagtttc cacagccatt gcctgtgtgt ggagacatca 120  
aggtggagtt cttccacaaa cagaacaaga tgctaaaaaa 159

<210> 246  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTENex8F sense

<400> 246  
caaaatgttt cacttttggg taaa 24

<210> 247  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTENex8R antisense

<400> 247  
taaaatttgg agaaaagtat cggtt 25

<210> 248  
<211> 226  
<212> DNA  
<213> Man

<400> 248  
gacaaaaatg tttcactttt gggtaaatac attcttcata ccaggaccag aggaaacctc 60  
agaaaaagta gaaaatggaa gtctatgtga tcaagaaaty gatagcattt gcagtataga 120  
gcgtgcagat aatgacaagg artatctagt acttacttta acaaaaaatg atcttgacaa 180  
agcaaataaa gacaaagcca accgatactt ttctccaaat ttttaag 226

<210> 249  
<211> 213  
<212> DNA  
<213> Chimpanzee

<400> 249  
atgtttcact tttgggtaaa tacattcttc ataccaggac cagaggaaac ctcagaaaaa 60  
gtagaaaatg gaagtctatg tgatcaagaa atcgatagca tttgcagtat agagcgtgca 120  
gataatgaca aggaatatct agtacttact ttaacaaaaa atgatcttga caaagcaaat 180  
aaagacaaag ccaaccgata cttttctcca aat 213

<210> 250  
<211> 212  
<212> DNA  
<213> Cattle

<400> 250  
tgtttcactt ttgggtaaac acattcttca taccaggacc agaggaaacc tcagaaaaag 60  
tagaaaaatgg aagtctatgt gatcaagaaa ttgatagtat ttgcagtata gagcgtgcag 120  
ataatgacaa ggaatatcta gtactcactt taacaaaaaa tgatctcgac aaagcaaata 180  
aagacaaggc caaccgatac ttttctccaa at 212

<210> 251  
<211> 211  
<212> DNA  
<213> Sheep

<400> 251  
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agaaaaatgga agtctatgtg atcaagaaat tgatagtagt ttgcagtata agcgtgcaga 120  
taatgacaag gaatatctag tgctcacttt aacaaaaaat gatctcgaca aagcaaataa 180  
agacaaggcc aaccgatact ttttctccaa t 211

<210> 252  
<211> 213  
<212> DNA  
<213> Goat

<400> 252  
atgtttcact tttgggtaaa cacattcttc ataccaggac cagaggaaac ctcagaaaaa 60  
gtagaaaatg gaagtctatg tgatcaagaa attgatagta tttgcagtat agagcgtgca 120  
gataatgaca aggaatatct agtactcact ttaacaaaaa atgatcttga caaagcaaat 180  
aaagacaagg ccaaccgata cttttctcca aat 213

<210> 253  
<211> 212  
<212> DNA  
<213> Red buffalo

<400> 253  
atgtttcact tttgggtaaa cacattcttc ataccaggac cagaggaaac ctcagaaaaa 60  
gtagaaaatg gaagtctatg tgatcaagaa attgatagta tttgcagtat agagcgtgca 120  
gataatgaca aggaatatct agtactcact ttaacaaaaa atgatcttga caaagcaaat 180  
aaagacaagg ccaaccgata cttttctcca aa 212

<210> 254  
<211> 213  
<212> DNA  
<213> Deer

<400> 254  
tgtttcactt ttgggtaaac acattcttca taccaggacc agaggaaacc tcagaaaaag 60  
tagaaaaatgg aagtctatgt gatcaagaaa ttgatagtat ttgcagtata gagcgtgcag 120  
ataatgacaa agaatatcta gtactcactt taacaaaaaa tgatctcgac aaagcaaata 180  
aagacaaggc caaccgatac ttttctccaa att 213

<210> 255  
<211> 214

<212> DNA

<213> Roe deer

<400> 255

```
atgtttcact tttgggtaaa cacattcttc ataccaggac cagaggaaac ctcagaaaaa 60
gtagaaaatg gaagtctatg tgatcaagaa attgatagta tttgcagtat agagcgtgca 120
gataatgaca aagaatatct agtactcact ttaacaaaaa atgatctcga caaagcaaat 180
aaagacaagg ccaaccgata cttttctcca aatt 214
```

<210> 256

<211> 213

<212> DNA

<213> Goitred gazelle

<400> 256

```
atgtttcact tttgggtaaa cacattcttc ataccaggac cagaggaaac ctcagaaaaa 60
gtagaaaatg gaagtctatg tgatcaagaa attgatagta tttgcagtat agagcgtgca 120
gataatgaca aagaatatct agtactcact ttaacaaaaa atgatctcga caaagcaaat 180
aaagacaagg ccaaccgata cttttctcca aat 213
```

<210> 257

<211> 213

<212> DNA

<213> Horse

<400> 257

```
atgtttcact tttgggtaaa tacattcttt ataccaggac cagaggaaac ctcagaaaaa 60
gtagaaaatg gaagtctatg tgatcaagaa attgatagta tttgcagtat agagcgtgca 120
gataatgaca aagaatatct agtactcact ttaacaaaaa atgatctcga caaagcaaat 180
aaagacaagg ccaaccgata cttttctcca aat 213
```

<210> 258

<211> 210

<212> DNA

<213> Dog

<400> 258

```
tttcactttt gggtaaacac attcttcata ccaggaccag aggaaacctc agaaaaagta 60
gaaaatggaa gtctatgtga tcaagaaatt gatagtattt gcagtataga acgtgcagat 120
aatgacaagg aatatctagt actcacttta acaaaaaaatg atctcgacaa agcaaatataa 180
gacaaggcca accgatactt ttctccaaat 210
```

<210> 259

<211> 213

<212> DNA

<213> Sun bear

<400> 259

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atgtttcact tttgggtaaa cacattcttc ataccaggac cagaggaaac ctcagaaaaa 60
gtagaaaatg gaagtctatg tgatcaagaa attgatagta tttgcagtat agagcgtgca 120
gataatgaca aggaatatct agtactcact ttaacaaaaa atgatctcga caaagcaaat 180
aaagacaagg ccaaccgata cttttctcca aat 213
```

<210> 260

<211> 210

<212> DNA

<213> Rabbit

<400> 260  
 tttcactttt gggtaaatac gttctttata ccaggaccag aggaaacctc agaaaaagta 60  
 gaaaatggaa gtctttgtga tcaagaaatt gatagtattt gcagtataga acgtgcagat 120  
 aacgacaaag aatatctagt acttacttta acaaaaaatg atcttgataa agcaaataaa 180  
 gacaaggcaa accgataact ttctccaaat 210

<210> 261  
 <211> 210  
 <212> DNA  
 <213> Hare

<400> 261  
 gtttactttt tgggtaaata cgttctttat accaggacca gaggaaacct cagaaaaagt 60  
 agaaaatgga agtctttgtg atcaagaaat tgatagtatt tgcagtatag aacgtgcaga 120  
 taacgacaaa gaatatctag tacttacttt aacaaaaaat gatcttgata aagcaaataa 180  
 agacaaggca aaccgatact ttctccaaaa 210

<210> 262  
 <211> 203  
 <212> DNA  
 <213> Antelope

<400> 262  
 acttttgggt aaatacattc ttcataccag gaccagagga aacctcagaa aaagtagaaa 60  
 atggaagtct atgtgatcaa gaaattgata gtatttgcag tatagagcgt gcagataatg 120  
 acaaggaata tctagtactc actttaacaa aaaatgatct tgacaaagca aataaagaca 180  
 aggccaacgg atacttttct cca 203

<210> 263  
 <211> 213  
 <212> DNA  
 <213> Kangaroo

<400> 263  
 tttcactttt gggtaaatac attcttcata ccaggaccag aggaaaattc agacaaagta 60  
 gaaaatggaa gtctttgtgg tgatcaagag attgatagta tttgcagtgc cgagcgatca 120  
 gataatgaca aggaatatct catactcaca ttatccaaaa atgatcttga caaagcgaat 180  
 aaagacaagg ccaaccgata cttttctcca aat 213

<210> 264  
 <211> 210  
 <212> DNA  
 <213> Python

<400> 264  
 tttcactttt gggtaaatac attcttcata ccaggaccag aggaaacctc agaaaaagta 60  
 gaaaatggaa gtctatgtga tcaagaaatc gatagcattt gcagtataga gcgtgcagat 120  
 aatgacaagg aatatctagt acttacttta acaaaaaatg atcttgacaa agcaaataaa 180  
 gacaaagcca accgataact ttctccaaat 210

<210> 265  
 <211> 208  
 <212> DNA  
 <213> Turkey

<400> 265  
 tcacttttgg gtaaatacat tcttcatagg actggatgaa aattcagaca aagtagaaaa 60

```

tggaagtcta gttgcagatc aggaacttga tggatatttc agtacagagc gctcagataa 120
tgacaaggaa tattttaatcc ttacattaac aaaaaatgat ctagacaaag caaataaaga 180
caaagccaac cgatactttt ctccaaat 208

```

<210> 266  
 <211> 213  
 <212> DNA  
 <213> Chicken

```

<400> 266
tttcaactttt gggtaaatac attcttcata ggactggatg aaaattcaga caaagtagaa 60
aatggaagtc tagttgcaga tcaggaactt gatgggtattt tcagtacaga gcgctcagat 120
aatgacaagg aatattttaat cttacatta acaaaaaatg atctagacaa agcaaataaa 180
gacaaagcca accgatactt ttctccaaat tta 213

```

<210> 267  
 <211> 210  
 <212> DNA  
 <213> Quail

```

<400> 267
ttcactttttg ggtaaataca ttcttcatag gactggatga aaattcagac aaagtagaaa 60
atggaagtct agttgcagat caggaacttg atgggtatttt cagtacagag cgctcagata 120
atgacaagga atattttaatc cttacattaa caaaaaacga tctagacaaa gcaaataaag 180
acaaagccaa ccgatacttt tctccaaatt 210

```

<210> 268  
 <211> 213  
 <212> DNA  
 <213> Goose

```

<400> 268
atgtttcact tttgggtaaa tacattcttc ataggactgg atgaaaattc agacaaagta 60
gaaaatggaa gtctagtgtc agatcaggaa cttgatggta ttttcagtac agagcgctca 120
gataatgata aggaatattt aatccttaca ttaacaaaaa atgatctaga caaagcaaat 180
aaagacaaag ccaaccgata cttttctcca aat 213

```

<210> 269  
 <211> 235  
 <212> DNA  
 <213> Trout

<220>  
 <221> misc\_feature  
 <222> (1)...(235)  
 <223> n = A,T,C or G

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<400> 269
gtttcacttt tgggtaaatn nnttctttgt ccctggacca gaggagaact ttgagaaggt 60
tgagaacggg acgttaccaa cggagacgtt accaacggcg acgttaccaa aggagcaggc 120
aggaaaccaa acgggaggaa cgggggacaa cgacaaggat tacctgatcc tctcactgac 180
aaagaacgac ctggacaagg ccaacaagga taaabcaaac cgatactttt ctcca 235

```

<210> 270  
 <211> 23  
 <212> DNA  
 <213> Artificial Sequence



<220>

<223> PTENex9F sense

<400> 270

gtgaagctgt acttcacaaa aac

23

<210> 271

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> PTENex9tga antisense

<400> 271

aaaaaaattc agacttttgt aatttg

26

<210> 272

<211> 194

<212> DNA

<213> Man

<400> 272

gtgaagctgt acttcacaaa aacagtagag gagccgtcaa atccagaggc tagcagttca 60  
acttctgtaa caccagatgt tagtgacaat gaacctgac attatagata ttctgacacc 120  
actgactctg atccagagaa tgaacctttt gatgaagatc agcatacaca aattacaaaa 180  
gtctgaattt tttt 194

<210> 273

<211> 180

<212> DNA

<213> Chimpanzee

<400> 273

gtacttcaca aaaacagtag aggagccgtc aaatccagag gctagcagtt caactttctgt 60  
aacaccagat gttagtgaac atgaacctga tcattataga tattctgaca cactgactc 120  
tgatccagag aatgaacctt ttgatgaaga tcagcataca caaattacaa aagtctgaat 180

<210> 274

<211> 176

<212> DNA

<213> Cattle

<400> 274

cttcacaaaa acagtagagg agtcatcaaa tccagaggct agcagttcaa cttctgtaac 60  
accagatggt agtgacaatg aacctgatca ttatagatat tctgacacca ctgactctga 120  
tccagagaat gaaccttttg atgaagatca gcatacaca attacaaaag totgaa 176

<210> 275

<211> 172

<212> DNA

<213> Sheep

<400> 275

cttcacaaaa acagtagagg agtcatcaaa tccagaggct agcagttcaa cgtctgtaac 60  
accagatgct agtgacaatg aacctgatca ttacagatat tctgacacca ctgactctga 120

cccagagaat gaaccttttg atgaagatca gcatacacia attacaaaag tc 172

<210> 276  
<211> 178  
<212> DNA  
<213> Goat

<400> 276  
tacttcacaa aaacagtaga ggagtcacat aatccagagg ctagcagttc aacgtctgta 60  
acaccagatg tcagtgcacaa tgaacctgat cattacagat attctgacac cactgactct 120  
gaccagaga atgaaccttt tgatgaagat cagcatacac aaattacaaa agtctgaa 178

<210> 277  
<211> 179  
<212> DNA  
<213> Red buffalo

<400> 277  
tacttcacaa aaacagtaga ggagccatca aatccagagg ctagcagttc cacttctgtg 60  
acacccgatg ttagtgacaa tgaacctgat cattatagat attctgacac cactgactct 120  
gatccagaga atgaaccttt tgatgaagat cagcatacac aaattacaaa agtctgaat 179

<210> 278  
<211> 179  
<212> DNA  
<213> Deer

<400> 278  
tacttcacaa aaacagtaga ggagtcacat aatccagagg ctagcagttc aacttctgta 60  
acaccagatg ttagtgacaa tgaacctgat cattatagat attctgacac cactgactct 120  
gatccagaga atgaaccttt tgatgaagat cagcatacac aaattacaaa agtctgaat 179

<210> 279  
<211> 173  
<212> DNA  
<213> Roe deer

<400> 279  
acttcacaaa aacagtagag gagtcacat atccagaggc tagcagttca acttctgtaa 60  
caccagatgt tagtgacaat gaacctgatc attatagata ttctgacacc actgactctg 120  
atccagagaa tgaacctttt gatgaagatc agcatacaca aattacaaaa gtc 173

<210> 280  
<211> 177  
<212> DNA  
<213> Goitred gazelle

<400> 280  
cttcacaaaa acagtagagg agtcacataa tccagaggct agcagttcaa cgtctgtaac 60  
accagatgtc agtgacaatg aacctgatc ttacagatat tctgacacca ctgactctga 120  
cccagagaat gaaccttttg atgaagatca gcatacacia attacaaaag tctgaat 177

<210> 281  
<211> 180  
<212> DNA  
<213> Horse

<400> 281

gtacttcaca aaaacagtag aggagccatc aaatccagag gctagcagtt caacttctgt 60  
aacaccagat gttagtgcaca atgaacctga tcattataga tattctgcaca ccaactgactc 120  
tgatccagag aatgaacctt ttgatgaaga tcagcataca caaattacaa agtctgaat 180

<210> 282

<211> 180

<212> DNA

<213> Dog

<400> 282

gtacttcaca aaaactgtag aggagccatc aaacccggag gctagcagtt caacttctgt 60  
gacgccagat gttagtgcaca atgaacctga tcattataga tattctgcaca ccaactgactc 120  
tgacccagag aatgaaccct ttgatgaaga tcagcacaca caaattacaa agtctgaat 180

<210> 283

<211> 177

<212> DNA

<213> Sun bear

<400> 283

cttcacaaaa acagtagagg agccatcaaa tcccaggagct agcagttcaa cttctgtaac 60  
accagacgtt agtgacaatg aacctgacca ttatcgatat tctgacacca ctgactctga 120  
tccagagaat gaaccttttg atgaagatca gcatacacaa attacaaaag tctgaat 177

<210> 284

<211> 177

<212> DNA

<213> Rabbit

<400> 284

tacttcacaa aaacagtaga ggagccatca aatccagagg ctagcagttc aacttctgta 60  
acgccagatg ttagtgacaa tgaacctgat cattatagat attctgacac cactgactct 120  
gatccagaga atgaaccttt tgatgaagat cagcatacac aaattacaaa agtctga 177

<210> 285

<211> 179

<212> DNA

<213> Hare

<220>

<221> misc\_feature

<222> (1)...(179)

<223> n = A,T,C or G

<400> 285

tacttcacaa aaacagtaga ggagccatca aatccagagg ctagcagttc aacttctgta 60  
acgccagatg ttagtgacaa tgancctgat cattatagat attctgacac cactgactct 120  
gatccagaga atgaaccttt tgatgaagat cagcatacac aaattacaaa agtctgaat 179

<210> 286

<211> 175

<212> DNA

<213> Antelope

012627-025.ST25

<400> 286  
acttcacaaa aacagtagag gagccatcaa atccagaggc tagcagttca acttctgttaa 60  
caccagatgt tagtgacaat gaacctgac attatagata ycttgacacc actgactctg 120  
atccagagaa tgaacctttt gatgaagatc agcatacaca aattacaaaa gtctg 175

<210> 287  
<211> 174  
<212> DNA  
<213> Varan

<400> 287  
ttcacaaaaa ccgtagaaga accatcaaac ccagaggcta gcagctcaac ttcagtaacg 60  
ccagatgtta gtgataatga acctgatcat tatagggtatt ctgataccac tgactctgat 120  
ccagagaatg aaccttttga tgaagatcag catacacaaa ttacaaaagt ctga 174

<210> 288  
<211> 175  
<212> DNA  
<213> Turkey

<400> 288  
ttcacaaaaa cagtagagga gccatcaaac ccagaggcta gcagttcaac ttctgttaaca 60  
ccagatgtta gtgacaatga acctgatcat tatagatatt ctgacaccac tgactctgat 120  
ccagagaatg aaccttttga tgaagatcag catacacaaa ttacaaaagt ctgaa 175

<210> 289  
<211> 182  
<212> DNA  
<213> Chicken

<400> 289  
ctgtacttca caaaaacagt agaagagcca tcaaatcccg aggctagcag ttcaacttct 60  
gtaacaccag atgttagtga caatgaacct gatcattaca gatactctga caccactgac 120  
tctgatccag agaatgaacc ttttgatgaa gatcagcata cacaaattac aaaagtctga 180  
at 182

<210> 290  
<211> 177  
<212> DNA  
<213> Duck

<400> 290  
cttcacaaaa acagtagaag agccatctaa tccagaggct agcagttcaa cttctgtaac 60  
gccagatgtt agtgacaatg aacctgatca ttatagatac tctgacacca ctgactctga 120  
tccagagaat gaaccttttg atgaagatca gcatacgcaa attacaaaag tctgaat 177